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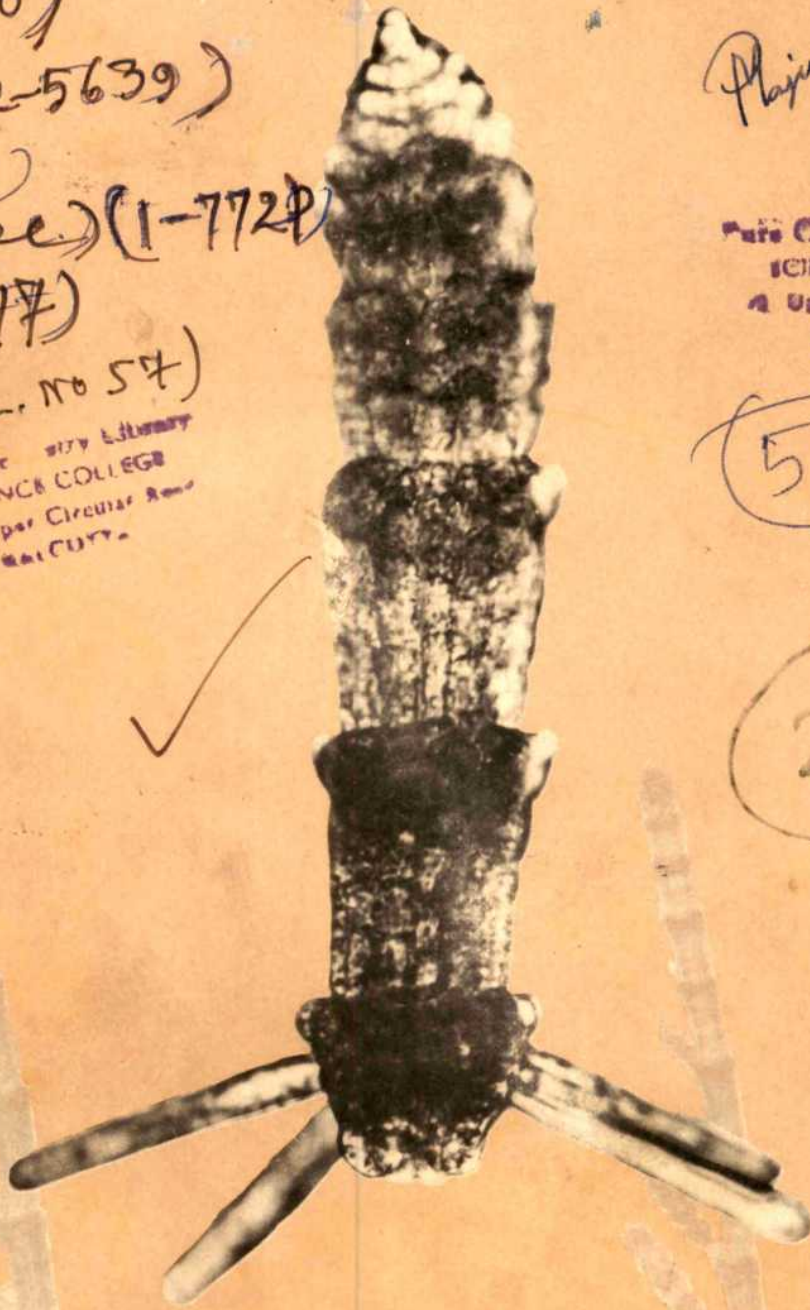
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The marine red alga *Centroceras*  
reproduces vegetatively by producing  
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laboratory, about 1890. A new bio-  
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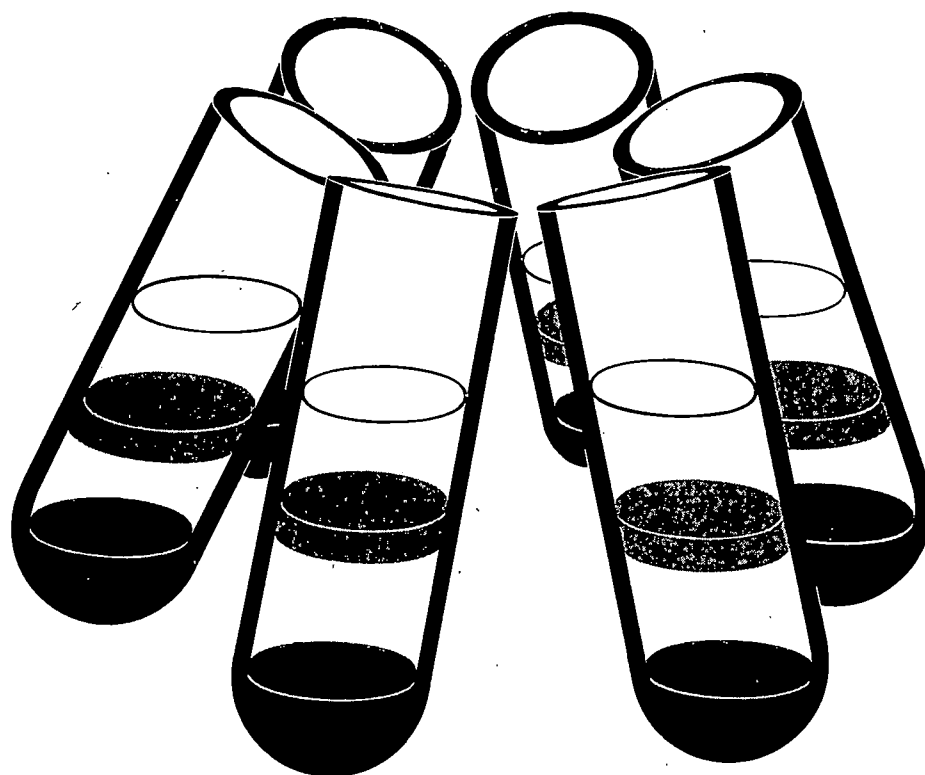
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Cover picture

*Locusta migratoria* eating *Sorghum*. Young *Sorghum* leaves are more palatable to locusts than older *Sorghum* leaves. The difference is related to the rate of release of HCN from leaves when bitten. See page 235.

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## Cover picture

A totally schematic illustration of  
how gene inserts can separate those  
DNA sequences that are represented  
in eukaryotic messenger RNA. Ex-  
perimental evidence for inserts in the  
ovalbumin gene is given on page 314  
and the subject is reviewed on page  
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A drainage ditch displaced by 250 cm  
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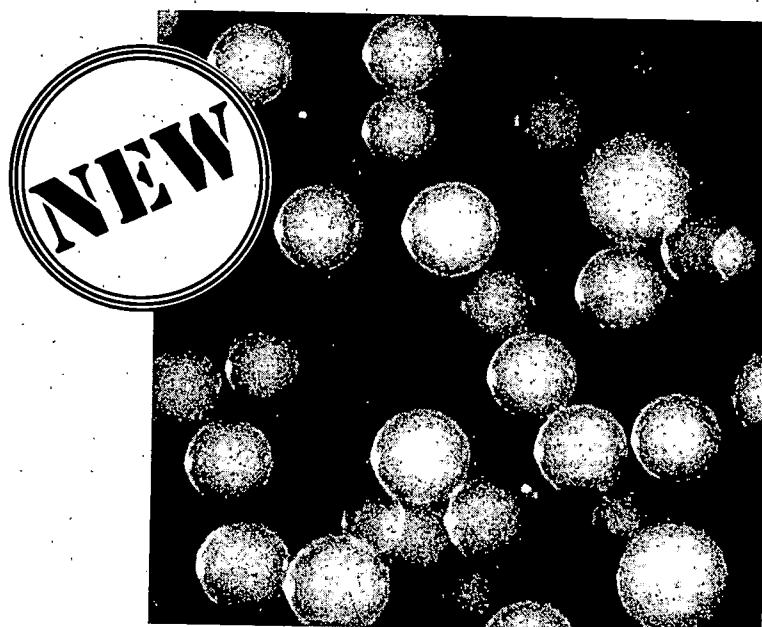
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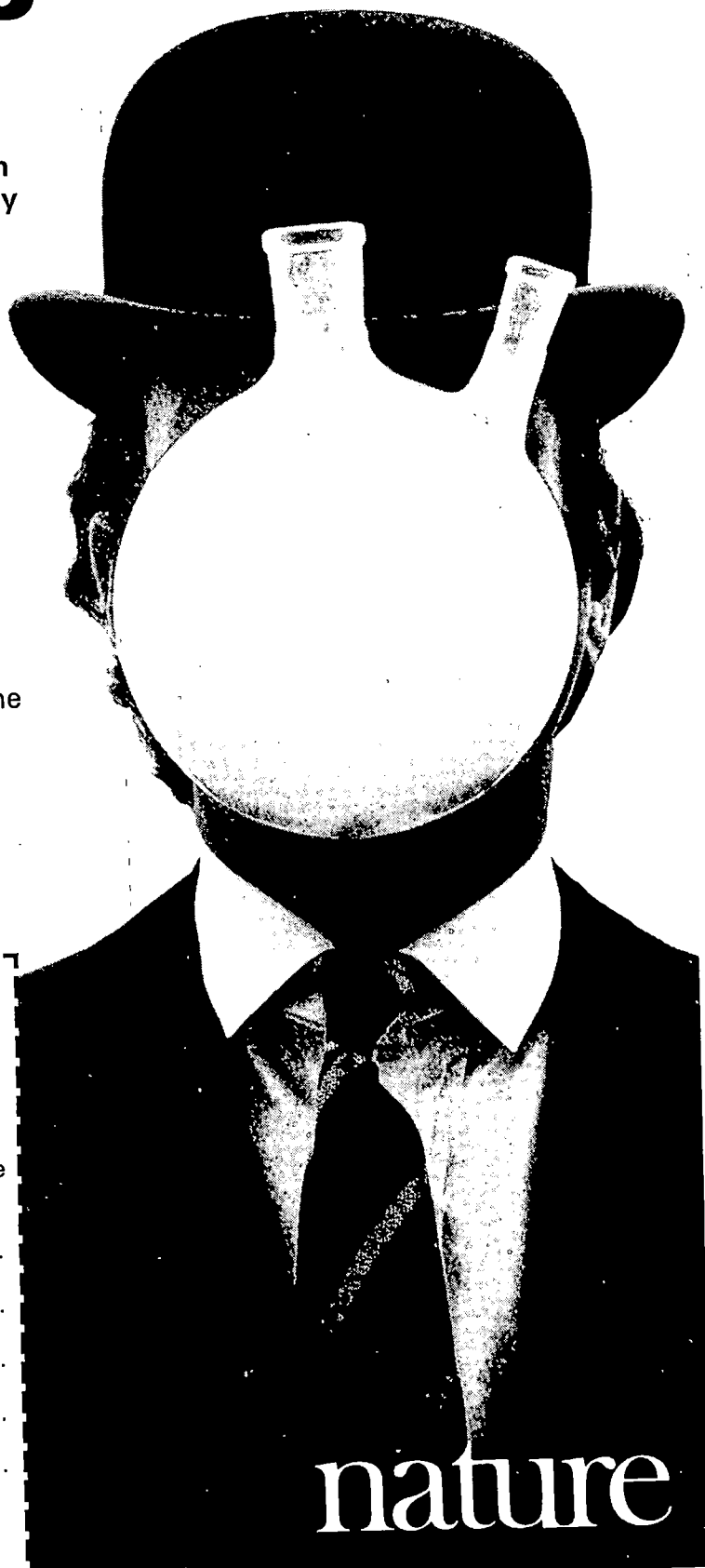
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**nature**

3 November 1977

## Sixty years of Soviet science

NEXT WEEK, on 7 November, the Soviet Union celebrates the sixtieth anniversary of the October Revolution. The discrepancy in dates, reflecting the change-over from Julian to Gregorian reckoning, neatly symbolises one of the major results of the Revolution—a commitment to implementing the results of modern scientific progress in the building of socialism.

For forty years, this policy was viewed from abroad as no more than an attempt to catch up with the burgeoning Western industrial economies. Soviet leaders' remarks about 'outstripping' the West were dismissed as mere propaganda—until the bleeps of *Sputnik 1* sent Western scientists running to their Russian primers and added to the information explosion an ever-increasing flood of cover-to-cover translations of Soviet journals.

One well-worn theme of Soviet writers on *naukovedenie* (the science of science) is that Soviet science must inevitably overtake the bourgeois variety, owing to its superior ideological basis. While we cannot support the idea that science, properly speaking, can belong to this or that political system, there does seem to be a distinct sociological phenomenon which may be termed 'Soviet science'—science carried out within and in the service of the Soviet State planning structure. Within this system science, like every other branch of production, is organised into neat five-year planning blocks. Research is envisaged officially as a search for 'solutions' to particular problems of the national economy, with the All-Union Academy of Sciences as 'coordinator for the whole scientific work of the country', and increasingly centralised control and 'unification' of academic institutions.

Unfortunately, it is precisely this centralised control which has led to major set backs for science in the Soviet Union, when high-level intervention by planners or ideologues overrode the scientists' desired research programmes. Lysenkoism, Stalin's attempt to ban quantum mechanics, the slow start in cybernetics (it appeared contrary to certain dicta of Marx and Lenin) are hardly a convincing argument for leaving decision-making in the hands of the State Planning Commission. Nor has this trend ceased. In recent years, the psycho-

logical theories of Snezhnevskii, who would attribute all mental disturbance and social nonconformity to schizophrenia, even when no schizophrenic symptoms are discernible, have begun to acquire the status of official dogma, and seem well on their way to repeating, in psychology, the disastrous course into which Lysenkoism led Soviet biology.

While the days are long past when a student might be asked to comment on the 'class significance' of a differential equation, ideological soundness remains a matter of utmost concern to the Soviet authorities. According to the latest (1976) regulations, a postulant for a higher degree must "combine a profound professional knowledge with a mastery of Marxist-Leninist theory and with the convictions of an active builder of Communist society". Official biographies and obituaries describe a scientist's minor Party offices as meticulously as his academic laurels. Organisers of international conferences know all too well that a Russian scientist of world repute will frequently fail to appear, being replaced by a junior colleague of mediocre scientific ability but greater Party commitment.

No one can deny the considerable achievement in Soviet science and technology over the last six decades, nor the fact that certain achievements, notably major irrigation projects, the space programme or the development of the resources of Siberia, would hardly have been possible without a centralised planning system. In the eyes of the comrade-in-the-street, science and scientists have acquired considerable charisma. Orders and decorations are regularly bestowed on deserving scientists by a grateful government.

Yet, by a typically Russian paradox, the constitutional position of scientists was until now anomalous in a State based officially on the union of workers and peasants. Only in the past month, with the publication of the revised constitution, has this been extended to read "workers, peasants, and intelligentsia". Recognised at last as part of the basis of the State, it is to be hoped that Soviet scientists will receive as an anniversary present a greater measure of freedom of movement and immunity from bureaucratic interference without which no science can effectively flourish. □

# The lipid hypothesis: orthodoxy by default?

John Rivers, a London-based nutritionist, looks at the debate over diet and coronary heart disease

Dr George Mann is a university teacher, a physician, a nutritionist and a heretic. He is one of the small number of outspoken opponents of the theory that the key to the modern epidemic of coronary heart disease (CHD) is the consumption of too much fat which is too high in cholesterol and has too low a ratio of polyunsaturated to saturated fatty acids. That this opposition should justify the term 'heretic' is not a reflection of the unchallengeable nature of the evidence supporting the 'lipid hypothesis', but the extent to which official gatherings of experts in different countries have made it national policy.

CHD is a sudden event, but as it only occurs in arteries narrowed and made less resilient by atherosclerosis, its origin must be sought in the factors which lead to years of deposition of cholesterol-rich atheromatous plaques. Virchow's observation over a century ago that the cholesterol in these plaques is derived from serum cholesterol suggested high serum cholesterol was the primary cause. Ample epidemiological evidence backs this view. The disagreement starts when one seeks the cause of high serum cholesterol.

As over 90% of our body cholesterol is endogenously synthesised, Mann dismisses dietary cholesterol as an important determinant of serum cholesterol. Rather, he suggests, one should look for factors which alter rates of synthesis or excretion. In his view the latter is crucial, and he notes a reduction in cholesterol breakdown is known to result from exposure to high levels of atmospheric carbon monoxide—hence the importance attached to smoking and pollution—and from consumption of *trans* unsaturated fatty acids.

Powerful evidence can be adduced to support his view that present dietary hypotheses of hypercholesterolaemia are mistaken. For example, a recent study of 2,000 subjects in Michigan found no significant differences in intakes of energy, total fat, unsaturated fat, saturated fat, or cholesterol between the upper and lower tertiles for serum cholesterol. What ultimately matters, however, is not the effect of diet on serum cholesterol but its impact upon mortality from CHD. Reviewing the dietary modification trials that have been conducted to date, Mann concludes that differences in mortality in primary or secondary clinical trials have yet to be shown. Although mortality from CHD is reduced in some, a diagnosis of the cause of death is relatively soft data, and the total death rate has remained constant. Mann argues that if CHD mortality really fell in these trials, mortality from something else must have risen for the total deaths to have been constant. He points out that this often seems to be cancer.

The idea that polyunsaturated fats predispose towards cancer dates from the studies of Pearce and Dayton (*Lancet* i, 464–467, 1974). Many nutritionists regard the idea as unproven—at the least the evidence about it is conflicting. But it is Morton's Fork. If, like Heyden (in *The Role of Fats in Human Nutrition*, Academic Press, 1975), one dismisses the apparent rise in cancer deaths as due to poor design of the trials, then the trials are also too poor to provide evidence about deaths from CHD. The trouble, says Mann, is that the experiments are not scientific. Scientists should design experiments to disprove hypotheses—dietary trials are still being done to confirm them. Because the lipid hypothesis is still not proven after 27 years, he says, the time has come for a rethink.

More troubling than Mann's scientific objections to the

lipid hypothesis are the effects that widespread acceptance of it has had. It has become self-sustaining: heretical opponents find it difficult to obtain research funds, and the medical hypothesis is being increasingly used as an advertising ploy—a massive vegetable oil industry needs evidence for the benefits of polyunsaturated fats.

Even more important, though, we may be doing exactly the wrong thing. Pursuit of the lipid hypothesis does not mean just swapping polyunsaturates for saturates. Polyunsaturates are too unstable, so much of the extra polyunsaturated fatty acids are provided by the substitution of soft margarines and stabilised vegetable oils for butter and animal fats. The trouble is, both changes lead to a dramatic increase in consumption of *trans* fatty acids, compounds whose known hypercholesterolaemic effect (Vergossen and Gottenbos, *The Role of Fats in Human Nutrition*) outweighs the putative benefits of polyunsaturated fats.

Butter contains 8% of its fatty acids as *trans* isomers, the product of biohydrogenation in the rumen. Vegetable oils, which like soft margarines are usually partially hydrogenated, contain *trans* fatty acid levels of up to 40%. Manufacturers of polyunsaturated oils and margarines may proudly state that they are high in polyunsaturates, but they do not state the levels of *trans* fatty acids, nor do they feel obliged to point out the crucial modification that those fatty acids may have undergone.

Like many of the critics of the lipid hypothesis, Mann has sought supporting evidence for his view from studies on the Masai. These meat- and milk-eating pastoralists have low serum cholesterol in spite of a high consumption of animal products. Mann argues that this can be expected—they have low levels of environmental pollution and eat little *trans* fatty acid. Nevertheless, his studies on 50 aortae obtained post mortem show that they do get atheroma but its effects are minimised by the tendency of the coronary artery to widen with age. This Mann attributes to heavy exercise, pointing to epidemiological studies which show a protective effect of heavy exercise. The question obtrudes itself. Is a dietary solution being sought because it is more acceptable than exercise?

It was sad that, to hear Mann talk, I had to attend a press gathering organised by the Butter Information Council, a public relations body representing the dairy industries of Europe. One must regret that he was not asked to present his views to a scientific audience, although they do have available a good summary in his latest paper (*New England Journal of Medicine* 297, 644–650, 1977). Our dietetic fate is in danger of being decided by a public relations battle between margarine salesmen and cowherds.

In the face of this, however, we cannot seek reassurance from the deliberations of the expert committees on the topic. Two successive UK committees on heart disease, under the aegis of the Department of Health and Social Security (*Diet and Coronary Heart Disease*, HMSO, 1974), and the Royal College of Physicians (*J. Roy. Coll. Physicians*, 10, 213, 1976), have come to diametrically opposed conclusions about the value of the lipid hypothesis. The evidence available to both was substantially the same. The latest report, from a US Senate Committee under Senator George McGovern, has recommended among other things a reduction by 30% in dietary total fat intake accompanied by a 50% rise in the consumption of polyunsaturated fatty acids. Such sweeping changes are only likely to be achieved by a substantial shift towards the use of vegetable oils and, according to Mann, may cause more problems than they solve. Not all that he says is convincing, but it is a powerful criticism of a view that is in danger of becoming orthodoxy by default. □



# Improving bench work

Recent legislation affecting employment in Sweden has implications for research, as **Wendy Barnaby** explains

**S**HOULD research be regulated by the laws that govern industry, or is research a special kind of activity needing a different set of laws? The question arises in Sweden as a result of legislation over the past couple of years to strengthen the employee's position in relation to the employer, and it has a good number of scientists worried. For although a few very small categories of employees are exempted, the laws apply to all sorts of working places, and these include universities, research institutes as well as factories, bureaucracies and businesses. Given the different needs of these organisations, the question arises whether it is possible to design one set of rules which will secure the employees' place within them and at the same time improve the quality of their output. It is becoming obvious that the answer is no.

The Law on Security of Employment, which came into effect on 1 July 1974, is the one with the most dramatic effects on research. Its basic tenets are that employment is to last until further notice, which cannot be given without reasonable cause. An employee who is dismissed can contest the dismissal in a court of law and is generally entitled to keep his job while the case is being decided. The law thus abolishes the traditional principle that the employer can fire employees as he sees fit. It also contains various rules about the order in which employees are to be laid off in times when there is no work for them to do (a 'last in first out' basis) and specifies that an employee laid off for this reason has first option on new jobs with his old employer for a year after his dismissal.

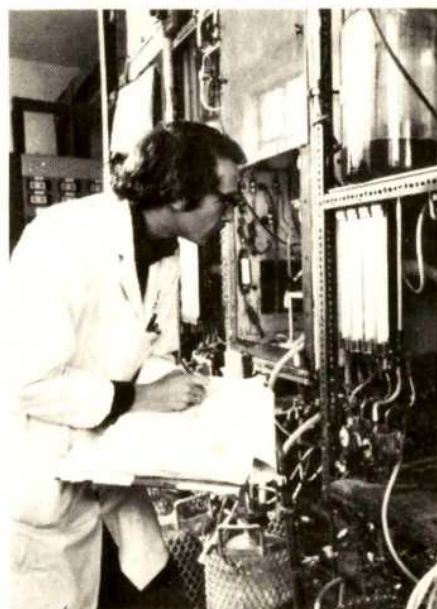
The Social Democrats' intention in passing this law was to protect lower-paid workers whose jobs were at the mercy of market trends. It is a long way from the conveyor belt at Volvo to university laboratories, yet the law applies to people working at both. In practice, it has meant that university lecturers have gained security in their jobs instead of having to re-apply for them annually or every third year. Only one category is still competitive, that of research assistant (*forskarassistent*), a position filled by a newly-graded PhD who has to re-apply for it after three years. The reasoning behind this exemption, according to a representative of the academics' union at one of Sweden's larger universities, is that possibilities should be kept open for

PhD's who want to go on researching and teaching.

On paper some flexibility has also been maintained with the prestigious position of docent, the last of the stepping stones to professorial rank. A docent's contract lasts for three years, after which time it may or may not be renewed. Because most docents are in their forties and fifties and have probably spent all their working lives at the university, it looked too callous to throw them out on to the street if they had become stale in their research. Under the law, therefore, the university is obliged to create for any docent whose contract is not renewed a new position at equivalent salary but not involving research. The funds for this are to come out of the university's research revenues. It is an extremely expensive way out, so expensive that it is hardly ever resorted to. In practice, docents have tenure.

Thus, after the statutory six months probation period, all members of a university department except research assistants have tenure, with docents' security practically assured. The results are not hard to imagine. No incompetents can be fired, so no new blood (except research assistants) can be hired. There is minimal circulation of staff and, many complain, a loss of creative atmosphere. The Rektor of Uppsala University, Professor Torgny Segerstedt, says there is an enormous risk that research will become very static.

Its effects will be felt in other ways, too. "I don't dare hire a new secretary", says one professor, "because I know I'll never be able to get rid of her if she turns out to be no good." A microbiologist complains: "I have a good research project I'd like to start, and I've even got a grant for it. But I can't hire a laboratory assistant. We could be stuck with him for ever". In theory, the law allows short-term contracts for specialists hired for a particular task, but in practice this may be impossible to enforce because it is difficult to identify a scientific project as a special job being done in an institute concerned with the same area of research. An assistant employed on, say, a two-year project who wants to stay at its completion would probably succeed in proving that he has taken part both in the specialised project and in the general work of the institute, and would then be considered to have been employed by the institute as



All affected:  
lab assistant to glass-blower



such.

Against defenders of the law who say that research council grants will keep some turnover in projects and personnel, it is argued that such grants will become just as tied up as ordinary projects because they are formally given to the universities which then pass them on to the successful applicants. When any project is over, therefore, the researchers could claim that it had been the university, not the Council, that had been employing them, and demand to stay. At this point the university would be obliged to see if it could find the displaced researcher a job he was competent to do, even if he was not enthusiastic about it.

Thus, a research biologist could be offered a job tutoring biology to first-year students, if such a vacancy were available. If no department had any suitable vacancy, the university would be entitled to fire him on the grounds of there being no work for him to do. In practice the university, acting totally within the law, has retained displaced researchers by firing the most-recently employed members of staff in jobs suited to the displaced researchers' qualifications. In small institutes, however, the regular staff normally dependent on research grants have to leave if their grants are not renewed.

It is difficult to see how, under the law, any research project could be abandoned while the researchers wanted to carry on with it. This raises questions about the whole direction of research: how to drop enquiries which are no longer interesting or profitable, and how to swing research into new channels. Problems would arise not so much at the end as at the beginning of new projects. Unless job security for skilled technicians can be provided, they won't be there in the first place.

#### Working life law

The other law making itself felt at research institutes is the Act on the Joint Regulation of Working Life (*Medbestämmandelag*, MBL), which came into force at the beginning of this year. Its purpose is to increase union participation in decision-making. It does this partly through revising the rules about decision-making, so that the management has to inform and negotiate with the union if any major changes are contemplated, and partly through widening the scope of issues about which the union is entitled to have a say. The most important provision in this respect is that unions and management who have a collective agreement on wages and conditions of work—which nearly all have—should negotiate agreements on joint regulation, which will set out the rights of the workers to organise and assign

work, hire and fire employees, and in general carry on the work of the organisation. The management is also required to provide the unions with information about financial and production matters and personnel policy.

Because most research establishments are financed by the government, the effects of MBL on research are somewhat modified by the Public Employment Act (*Lag om Offentlig Anställning*), which is designed to give public employees the benefits of MBL without trespassing on that part of their work which, because of democratic norms, the public at large has the right to preside over. As far as research institutes go, this means that the employees would not, for example, be able to change the general focus of research as laid down by parliament in the institute's statutes. It also means that, although the management must inform and negotiate with the union, it is not obliged to accept union opinions in questions considered to be in the public domain. In the absence of agreements on joint regulation, MBL in research institutes is at the moment largely a matter of an increase of information and negotiation. But the new system does take up a lot of time.

In some institutes, the atmosphere has been poisoned by union-management disagreements over how MBL should work. "Academic unions are young and inexperienced in comparison with industrial unions", says one physics professor who has had a hard time of it. "They minimise the role of informal contacts and consultations which are so important in industrial negotiations". Although running-in problems are giving some of

the unions a bumpy ride, all agree that their power has increased. "Research is expensive", comments one employee, "and if everybody has to take part in all decisions and planning it will cost more money, time and paper. With the help of MBL, employees might insist on comfort in laboratories instead of equipment. On the other hand, MBL based on public opinion might enforce the financing of research which might otherwise not be carried through".

Different disciplines envisage different problems. "Take an experimental team", says another physics professor. "It is made up of, say, a professor, an assistant professor, a PhD, a couple of graduate students and some technicians. It is a small group and its members usually get together and decide on working practices that suit them all, which helps to mobilise creativity and enthusiasm. But the members of these groups are represented by different unions, who are not involved in the immediate situation but who will now decide on what is to happen there. Direct democracy is being substituted by indirect, and it won't help the atmosphere in the laboratories".

Both laws are quite new, and their full impact on research may not be clear for a few years yet. What does seem obvious is that, especially in the case of the Security of Employment Act, it does research little good to be swept up with industry and put into the same legal basket. Some see Swedish research being irreparably damaged. The government is aware of the difficulties and is to conduct a review. In the meantime, Sweden will remain a focus of attention for external as well as internal interests. □



Uppsala University, Scandinavia's oldest



## USA

## Security conscious

*The Worldwatch Institute, the private Washington-based research organisation concerned with global problems, published its latest pamphlet last week. Chris Sherwell reports*

"THE present deficiency of assured energy resources is the single surest threat that the future poses to our security and to that of our allies", the US Secretary of Defense, Harold Brown, told a gathering of business leaders in the United States last week. For a namesake of his at the Worldwatch Institute, the timing was near-perfect. Lester Brown, Worldwatch's president, was about to release *Redefining National Security*, which considers why energy and other global problems pose a new threat to national security that only a new approach can combat.

These new threats to security, Brown says, "arise less from the relationship of nation to nation and more from the relationship of man to nature". From his discussion of them he concludes that the traditional military concept of national security is no longer adequate for an interdependent world facing global ecological and economic problems. Political leaders have to realise that national security is meaningless without global security; countries must address the problem, he says without amplification, "cooperatively".

### OTA appointment

THE position of Director of the Congressional Office of Technology Assessment (OTA) has been given to Dr Russell W. Peterson. He is president of a lobbying organisation concerned with global issues called New Directions and formerly chairman of the Council of Environmental Quality and Governor of the State of Delaware.

The choice follows several months of speculation and political infighting sparked off by allegations that Senator Edward Kennedy was trying to take control of the OTA and had engineered the resignation of its founder and first Director, Emilio Daddario. These allegations were strongly contested at the time and will be further diminished in credibility by the fact that Peterson is a Republican with a reputation for independence. In addition, Kennedy has been scrupulous in avoiding any appearance of interference in the selection process.

Other nominees for the job were Russell Train, former head of the Environmental Protection Agency, Daniel Desimone, presently the deputy director of OTA, and John Sawhill, former head of the Federal Energy Administration.

Sandy Grimwade

The discussion itself ties together several well-known threads of argument that have already appeared in other Worldwatch publications. Thus, in respect of energy, Brown says that an oil-dependent world, in a nuclear limbo and facing climate alteration with heavy use of coal, must act in concert to produce a timetable for a transition to renewable resources. The world's biological systems, on which the global economy depends, says Brown, are similarly threatened by excessive human claims: fishery catches exceed the long-term sustainable yield, tree-cutting exceeds the regenerative capacity of forests, grasslands deteriorate as livestock and human populations increase, and erosion damages croplands as population pressures mount.

Brown takes his arguments further. Humans can inadvertently or intentionally alter global climatic patterns, he states, causing agricultural output to shrink and adversely affecting the survival prospects of hundreds of millions of people. And a "basic transformation" in the world food economy in the 1970s means that the global balance between demand and supply remains delicate. This, says Brown, is attributable to agricultural shortcomings in Eastern Europe and the USSR and agricultural mismanagement elsewhere, rapid population growth and "negative ecological trends"—deforestation, overgrazing, desert encroachment, soil erosion and flooding.

Finally he argues that economic threats to security—simultaneous inflation and unemployment on a global scale—aggravate social divisions, not least through a worsening income distribution. Taken with the other factors, which also translate into economic stresses, the military's role in securing a nation's well-being and survival is "relatively less important than it once was". But political leaders "perceive the new threats dimly".

Outside the optimists who through faith in man's ingenuity see no problems ahead, doubts about all these arguments will come from those who believe ecological and environmental problems will resolve themselves at great human cost regardless of political intervention, and from those who believe in political intervention but have little hope for it. Doubts of the first kind may simply be unacceptable to Brown, but the second kind pose a difficulty. After all, is it really the case that if *all* nations don't hang together they will hang separately? □

## BRITAIN

## Help at the margin

*UK Research Councils have received a small windfall. Chris Sherwell reports*

WHEN the UK Advisory Board for the Research Councils (ABRC) holds its regular meeting at the end of next week, it will consider how to advise Shirley Williams, the Secretary of State for Education and Science, on the allocation of an extra £4 million among the country's research councils. The sum was added to the department's 1978-79 Science Budget by the Chancellor of the Exchequer, Mr Healey, in his 'mini-budget' last week. Science administrators who recognise, in the words of one, that "it is the margins which cause us the misery", greeted the news enthusiastically.

It is not yet known whether the extra cash represents a once-and-for-all increase or the beginning of a longer term upturn. No indication of what precisely will be happening in the period after 1978-79 is yet available. This means that research councils must prepare options: greater expenditure on capital projects or special programmes will make different demands from improved research grants, for example, which would be recurrent. Last year's forward look means that a fair amount was already known of what councils might want to do with the extra cash.

Mrs Williams has asked the ABRC for its "urgent" advice. If a decision is not made next week, the next meeting is set for 9 December. □

## NETHERLANDS

## Limited progress

*The position regarding recombinant DNA research in the Netherlands is still not clear. Casper Schuurings reports*

THE old Dutch government, still in office five months after the 25 May elections, has decided to start preparing legal rules for recombinant DNA research in the Netherlands. The minister of Health and Environmental Protection, in a letter to parliament, says that so long as not enough is known of the positive and negative consequences of this research, no concrete judgment can be given about the contents of such legislation. "There is also not enough known about the social relevance of this research and therefore of the priority it must have in research generally". In the meantime,

he added, the greatest possible reserve should be exercised.

Practising scientists have expressed their disappointment and concern over this lack of clarity about the policy the government foresees. They fear that the degree to which the Netherlands is falling behind in this field will become greater, and there is concern about the matter of contracts between researchers, their universities or institutes and the Commission on Genetic Engineering of the Royal Netherlands Academy of Sciences. The Commission published a report on 30 March which concluded that recombinant DNA research should be developed with stringent precautions and according to clear regulations contained in legislation which should be implemented quickly.

The government is not following this advice exactly, but the signing of research contracts has not ceased. Researchers at the Free University of Amsterdam signed a contract with their own board and with the commission, and the group is about to start work. Safety control remains with the university itself. Groningen University has also signed a contract, but is further from starting.

The commission's report proposed two commissions, one concerned with safety measures, the other with ethical aspects. The minister, however, announced a new and broader *ad hoc* commission to take over a part of the task of the Academy's commission, which is now composed only of experts. This *ad hoc* commission has to register, advise and indicate the potential dangers of the research in different categories of risk.

The League of Scientific Researchers last month reacted critically to the report, saying it "underestimated and played down" the risks of recombinant DNA research. "The safety measures proposed by the commission are suggested more by their feasibility than by their demonstrated safety", the league said, and praised the minister, who will not return in the new cabinet, for perceiving the need for a broad discussion and for a broader commission. The league recommends a "freezing" of the experiments, and urges the government to stimulate discussion involving scientists, politicians, the lay public and others.

No requests have come so far from industry to start recombinant DNA research. Unilever announced earlier that it was making preparations, and Gist-Brocades is known to be doing the same. But union members are unhappy about this. One of the largest unions in the country has written to three ministers requesting a stop to the research while no legislation exists.

## COMECON

● Protests against the US decision to produce neutron bombs have occupied an important place in the Comecon media over the past three months, with special emphasis being placed on protests by scientists or scientific bodies. Not surprisingly, such protests have so far come from the scientific establishment, but more recently even 'dissident' opinion was called in as support, with an interview on Prague radio with Academician Andrei Sakharov in Moscow.

Sakharov's opposition to nuclear warfare is well known. Early in his career of protest he turned over his savings from his work on the Soviet hydrogen bomb to cancer research. It was his apprehension of the danger of nuclear catastrophe which led to his first major *samizdat* essay, *Progress, Coexistence and Intellectual Freedom*, in 1968. As a Nobel Peace prize-winner, Sakharov would surely have been approached even earlier in the current campaign were it not for his reputation of dissidence.

The interviewer attempted to dissociate 'Sakharov the Soviet scientist' from 'Sakharov, the number two Soviet dissident', alleging that in this second *persona* Sakharov merely rubber-stamped the views of his wife—the implication being that while she was in Italy for medical treatment, people could witness the 'real' Sakharov. But he refused to comment on the neutron bomb, save in the context of nuclear armament as a whole. The question of this one particular weapon, he said, must not be misused for propaganda purposes and must not be given moral and emotional overtones. He also suggested that it was fear of a (conventional) Soviet attack on Western Europe which had led the USA to work on the neutron bomb, and that, although convinced that neither the Soviet Union nor any nation wants a war, twice in this century wars had broken out "irrationally".

This latter statement, which contradicts the classical Marxist interpretation of twentieth century history, was characterised by the interviewer as "ludicrous". Sakharov himself was said to bear the "evil marks" of dissident thinking. But in spite of this and Sakharov's refusal to take action against one specific weapon, the interview was broadcast and presented as something of a journalistic scoop for the current anti-neutron bomb campaign.

● *Interkosmos* 17, launched on 24

September, inaugurated a new generation of Comecon space probes. Previous *Interkosmos* craft had been small, relatively light satellites used for limited studies of cosmic and solar radiation, the magnetosphere and ionosphere. The satellites were not properly stabilised with respect to the Sun, and hence the data obtained was only of a fairly general type.

Accordingly, a new type of satellite, called an "automatic general-purpose orbital station" (AGPOS) was developed for the *Interkosmos* programme. A prototype was launched last year as *Interkosmos* 15; *Interkosmos* 17 is the first operational AGPOS. Designed for long-term automatic operation, the AGPOS includes a special orientation system and can even be equipped with an orientation platform to carry instruments for the high-accuracy investigation of solar flares.

Although the *Interkosmos* programme theoretically includes all countries of Comecon, six out of the twelve on-board experiments were provided by Czechoslovakia, including a laser reflector to determine the distance between the satellite and earth, a silicon cosmic-ray detector, and devices to measure the mass and velocity of meteorites. Czechoslovakia is playing an increasingly large role in the *Interkosmos* programme. Czech trainee-cosmonauts preparing for the manned *Interkosmos* programme are reported to have completed their theoretical work and are training in simulated conditions. One of them is expected to go on a mission with a Soviet cosmonaut in 1978.

In addition to participating in *Interkosmos*, Czechoslovakia has also provided experiments for the Soviet *Prognoz* 4, 5 and 6 satellites which have highly elliptical orbits. The launching of an independent Czechoslovak satellite, and the inclusion of Czech instruments on Soviet lunar and interplanetary probes is also envisaged.

At the same time Bulgaria, hitherto a relatively minor participant in *Interkosmos*, is also planning an independent space programme to be associated with the 1,800th anniversary of Bulgarian statehood next year. A recent decree of the Council of Ministers approved a regulation introducing "a nationwide programme to study and utilise outer space, and a preparatory programme to launch into outer space the project 'Bulgaria 1300'".

Vera Rich



## IN BRIEF

**Food action urged**

A publication from Earth Resources Research, the research arm of Friends of the Earth in Britain, urges government action using taxes and subsidies to encourage consumption and production of foods that strengthen consumers' health, minimise environmental pressures and make the best use of food resources.

*Changing Food Habits in the UK*, by Chris Wardle, argues that the two main government regulatory bodies, the Food Standards Committee and the Food Additives and Contaminants Committee, should be provided with their own scientific staff and research facilities. There should also be, he says, an intensive programme of consumer education on nutrition, the introduc-

tion of nutrition labelling, and an independent body to oversee all forms of food advertising.

**German technology assessment?**

The opposition CDU/CSU parties in the West German Bundestag have proposed a motion to set up an office of technology assessment. They proposed this once before, in 1972. The new motion follows years of discussion and calls for the office to be set up within the existing Bundestag administration and for expert knowledge to be gained by placing orders both at home and abroad. It is regarded as important that Federal government offices should call on foreign experts for advice. The Bundestag will deal

with the motion over the next few months.

**Europe compact suggested**

Professor Peter Odell, appointed earlier this month as a part-time consultant to the UK Department of Energy, said this week that the restraint on oil and gas development imposed by the lack of a West European agreement should be overcome by a compact between the exporting and importing countries concerned.

In a joint paper with Dr K. Rosing, his colleague at Erasmus University, at an Institute of Fuel conference, he said that Europe's strategy for oil and gas production is "constrained more by politics and institutions than it is by the likely size of the resource base".

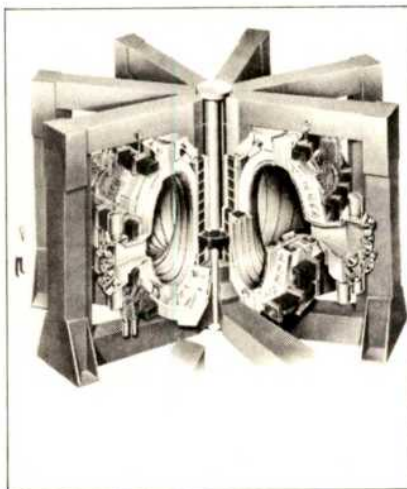
THE EEC's Joint European Torus (JET), which was finally given to the UK's Culham Laboratory last week, is essentially a device to confine the ionised particles of a plasma long enough for fusion reactions to occur between them. The two light elements most suitable for use in fusion machines are deuterium and tritium. The former is plentiful—it can be extracted from seawater—but the latter does not occur naturally and is made by bombarding lithium, a naturally occurring element, with neutrons.

The most successful machine designed so far for confining plasma, the tokamak, was developed in the USSR and forms the basis of the JET design. The plasma is confined within an evacuated D-shaped torus by a magnetic field, created by field coils linking the torus, and by a current flowing through the plasma. For the energy obtained to be greater than that put in, plasma temperatures have to be about 100 million °C and the product of plasma density and confinement time has to be greater than  $10^{14} \text{ cm}^{-3}\text{s}$ .

It is the attainment of such stringent operating conditions that has made fusion so difficult to harness. JET aims to achieve higher temperatures and plasma densities and longer confinement times than any of its predecessors. According to the original plan it was to take five or six years to build. Experiments were then to proceed for two to three years, by which time they would have produced good enough results for a prototype reactor to be designed.

There is doubt, however, about the realism of this timescale. In spite of progress so far, it is unlikely that

the road to what has been called a cheap, inexhaustible and clean source of energy will be smooth. As well as technical problems, those of fuel

**Fusion's promise****BACKGROUND**

supply, waste disposal and potential radiation leaks will have to be tackled, even though these may not be so great as those for fission reactors.

The radiation hazard stems from the possible release of tritium, a radioactive gas which will have to be contained within the reactor. Tritium for the fusion process will be manufactured as hot neutrons interact with lithium in the container walls. Constant bombardment of the walls will make them radioactive and wear them out so that they will have to be replaced from time to time. Even

though the products of the fusion process will not be radioactive and will pose no disposal problem, the old reactor walls will. The fuel supply problem will arise when world reserves of lithium run low. Present estimates put the reserve at about the same as uranium.

The enthusiasm for fusion as the answer to the world energy problem is based on the knowledge of what it could do, theoretically and not on what it actually might do on a large scale. If the fusion reaction were between deuterium and deuterium, for example, the problem of lithium supply would vanish. If it were between deuterium and helium-3 an added advantage would be that neutrons would not be produced and the problem of damaged radioactive walls would vanish. Similar advantages would be gained by using hydrogen and boron. But all these alternative fuels have their drawbacks when it comes to operating a large scale fusion machine, mainly because they would need even higher temperatures to ignite fusion reactions. Deuterium and tritium are at present the only promising fuels.

The outlook for JET itself, however, is fairly bright considering the two years delay. While politicians were negotiating, the team at Culham was building one-off pieces of equipment and refining the design. It has already placed phased contracts for some of the larger parts including the coils for the toroidal field. The next step is to appoint a head of project and management committee. Firm contracts for the rest of the parts will be placed throughout Europe and construction should begin within a few months.

# correspondence

## Petition for Argentinian scientists

SIR,—Some colleagues in our cancer centre are very concerned by the actual situation in Argentina and we have decided to propose the adjoining text for the approval of the international cancer community.

The undersigned cancerologists are very concerned by the situation in Argentina where personal security is increasingly threatened, fundamental rights are denied and where repression and arbitrary police action severely effect the scientific community ('Repression in Argentina: scientists caught up in tide of terrors' *Science* **194**, 1397; 1976; and 'More on Argentina' *Nature* **263**, 452; 1976).

These cancerologists refuse to participate under such conditions in the twelfth International Cancer Congress, which is scheduled for 5–12 October 1978 in Buenos-Aires, and ask all the members of the international scientific and medical community to join them in their refusal.

The undersigned also refuse to participate in meetings organised in any country subject to police repression and where the rights of man are systematically violated.

We invite physicians and scientists who share these opinions to join us in this protest by signing this petition. Copies of the petition will be made available on request to Dr J-C. Salomon, Institut de Recherches Scientifiques sur le Cancer, CNRS, B.P. 8, F 94800 Villejuif, France.

A similar action is underway among United States cancerologists.

LOUISE HAREL

JOSE URIEL

JEAN-CLAUDE SALOMON

Villejuif, France

## Exchange agreement

SIR,—I was glad to see Vera Rich's article (13 October, page 553) about the new exchange arrangement between the Royal Society and the Academy of Sciences of the USSR, but sorry to note that it contained several misleading statements. Firstly, the seven senior and four junior visits she referred to are in the *current* agreement, and are replaced in the new agreement, to come into effect on 1 April 1978, by a more flexible arrangement for a total of 49, not 40, man-months. The Royal Society hopes that this number will be increased and that the more flexible exchange agreement will lead to wider support for visits in both directions.

Vera Rich's comparisons with UK–Poland exchanges are also highly misleading as the figures she quotes for UK–USSR visits refer only to those under the Royal Society–Academy exchange agreement and do not refer to many visits under other auspices, while those for UK–Poland are evidently all-inclusive; Royal Society–Polish Academy visits account for only about ten each way.

R. W. J. KEAY

The Royal Society,  
London, UK

## Which diet?

SIR,—Colin Blythe and Howard Rush in their article 'What kind of food policy?' (4 August, page 386) mention the confusing advice available "to individuals who wish to follow sensible eating regimes".

Over past years ever-increasing doubt has been cast upon the validity of the theory that animal fats and cholesterol in human food cause arteriosclerosis and its resultant disorders. Even drastically reducing fat consumption and substituting animal fats to a large extent with distasteful plant oils of high polyene acid content, does not restore blood cholesterol levels to normal, nor bring about a retrogression of arteriosclerosis.

The fat theory offers us no explanation of the fact that in primitive populations, whose diet contains a very high proportion of animal fats, the incidence of arteriosclerosis and abnormal blood fat levels is practically nil. The diet of such people (Massai, other nomadic tribes, Eskimos) usually contains very little carbohydrate. Might is not therefore be the carbohydrates and not the fats that are responsible for the cardiovascular disorders connected with other types of civilisation?

Support for this idea is provided by observations on the cholesterol levels in

subjects of various age groups on a low-carbohydrate diet (70 g daily). Data collected from over 300 individuals have revealed that the cholesterol can be brought down by 50 mg% in 39% of the patients over 60 years of age, in 52% of those between 40 and 60, and in 67% of the group under 40. In the under-forties, restriction of carbohydrate intake can bring about permanent normalisation of the values within a short period of time, if we consider the average values as shown in the figure.

The possibility exists that every case of hypercholesterinemia could be reversed, or even avoided in the first place, if a low-carbohydrate diet were to be adopted in time. If we were to consume as little carbohydrate as the above mentioned primitive peoples, hyperlipidaemias and arteriosclerosis would probably not occur. Such disorders develop over the years under the influence of unphysiological quantities of carbohydrate (and their reversibility declines with advancing years) until the point is reached at which animal fats and cholesterol in the food bring about a rise in blood cholesterol levels. The efficacy of a low-carbohydrate diet in reducing overweight and in correcting metabolic disorders has already been established. Perhaps it would now be worthwhile considering the substitution of the fat theory by a carbohydrate theory in explaining the diseases connected with human civilisation.

WOLFGANG LUTZ

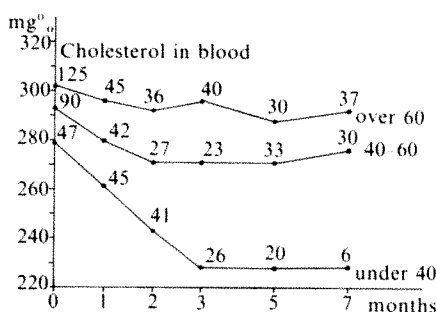
Facharzt für Innere Medizin,  
Salzburg, Austria

## On missing the point

SIR,—It is surprising that Bowne (13 October, page 556) finds some research ridiculous since it involves the discussion of polygenes, which are "unknown". It is surely a basic part of the scientific method to describe observed phenomena (familial resemblance for metrical characters) in terms of abstract entities (polygenes). Current work in genetics is not analogous to von Leeuwenhoeck studying ribosome structure, since the phenomena accessible to him did not suggest the idea of the ribosome.

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# news and views

## Aether drift detected at last

from Michael Rowan-Robinson

A GROUP of experimenters from Berkeley (Smoot, Gorenstein & Muller, *Phys. Rev. Lett.* **39**, 898), using a U-2 aircraft in the unfamiliar mode of looking upwards, seem to have decisively detected the motion of the Earth with respect to the cosmic microwave background. This radiation, with the spectrum of a 2.7 K blackbody, is believed to be the relic of the 'fireball' (radiation-dominated, optically thick) phase of the Universe. The isotropy of the microwave background to one part in  $10^3$  has been the strongest argument in favour of the homogeneous, isotropic models of the Universe dreamed up by Einstein, de Sitter, Friedmann, Eddington and Lemaître in the 1920s. But it has long been expected that a simple dipole anisotropy of order  $10^{-4}$  to  $10^{-3}$  should be found, due to the random motions that galaxies have with respect to each other and to the cosmological frame of reference (the frame in which the expansion of the Universe looks exactly isotropic, according to the models). The radiation should look slightly hotter in the direction we are travelling towards, and slightly colder in the direction we are travelling from, by an amount  $\Delta T/T \approx v/c$ , due to the Doppler shift. Failure to detect this effect would put us in the uncomfortable position of happening to be exactly at rest with respect to the cosmological frame. It was Michelson and Morley's failure a century ago to detect a contribution from the motion of the Earth to the local velocity of light that led to the downfall of aether theories and ultimately ushered in the relativistic age. A completely isotropic microwave background might have been equally revolutionary, but the Berkeley workers seem to have saved us from this fate. However the magnitude of the velocity deduced for the Milky Way,  $600 \text{ km s}^{-1}$ , is so large as to

throw existing ideas about our cosmic environment into disarray.

The Berkeley group did their experiment in the NASA-Ames Earth Survey U-2 aircraft and made eight flights over a period of 5 months. Two radiometers working at a frequency of 33 GHz ( $\lambda=9 \text{ mm}$ ) point at positions  $60^\circ$  apart on the sky,  $30^\circ$  on either side of the zenith, and the difference between the signals in the two receivers is recorded. An isotropic background would therefore give zero output wherever you looked. The plane flies on a level path, banking and reversing its direction of flight every 20 min. The whole apparatus is rotated through  $180^\circ$  every 64 s in order to eliminate asymmetry between the two radiometers. Anisotropy in the Earth's atmospheric background is monitored with a second receiver working near the strong oxygen line at 5 mm. The effect of any rapid changes in the gain of the radiometers is reduced by rapid switching between them at 100 Hz.

About 5% of the sky was sampled, all in the Northern Hemisphere. The results agreed well with what would be expected due to motion of the Earth: a temperature excess in one direction, a deficit in the opposite direction, and in between a cosine dependence on the angle between the direction of the peak and the direction of observation. The derived velocity, after correction for the motion of the Earth round the Sun and other minor effects, is  $390 \pm 60 \text{ km s}^{-1}$  towards a direction R.A. (Right Ascension)  $= 11.0 \pm 0.5 \text{ h}$ , declination (dec.)  $= 6^\circ \pm 10^\circ$  (galactic longitude ( $l$ )  $= 248^\circ$ ; latitude ( $b$ )  $= 56^\circ$ ). This is within  $2\sigma$  of the recent preliminary ground-based measurements of Corey & Wilkinson (*Bull. Amer. Astr. Soc.* **8**, 351; 1976), which are of lower accuracy. When corrected for the motion of the Solar System round the Galaxy, the net velocity of our Galaxy with respect to the microwave background is  $603 \text{ km s}^{-1}$  in direction R.A.  $= 10.4 \text{ h}$ , dec.  $=$

$-18^\circ$  ( $l=261^\circ$ ,  $b=33^\circ$ ). Apart from this dipole anisotropy the microwave background is isotropic to one part in 3,000 and limits can be set on the rotation of the Universe ( $<10^{-9}$  arc s per century) and on the average energy-density of long-wavelength gravitational radiation (less than the so-called "critical" density which would close the Universe).

What are we to make of this? The authors note that the velocity they have found conflicts with various attempts to measure our velocity with respect to nearby galaxies, but offer no explanation of this. With respect to the Local Group of galaxies, the motion of the Solar System hardly differs from that expected due to our circular motion round the Galaxy. This suggests that the whole Local Group has to be moving along together at this velocity of  $600 \text{ km s}^{-1}$  with respect to the microwave background. And this velocity is more than ten times the residual random motion of galaxies within 20 Mpc about the Hubble flow, so that most nearby galaxies, including the Virgo cluster of galaxies, would seem to have to move along together at this velocity. The Universe may be much more inhomogeneous than we have realised till now, and we may have to be careful about interpreting the expansion time-scale we measure locally as the age of the Universe.

The Berkeley measurement also conflicts with the velocity of the Earth with respect to more distant galaxies (at several hundred Mpc) determined by Rubin *et al.* (*Astr. J.* **81**, 687; 1976). They also found a velocity of about  $600 \text{ km s}^{-1}$ , but in a direction almost at right angles to the velocity with respect to the background. This 'Rubin-Ford effect' may not seem so surprising now. The matter in the Universe may be divided up into quite large 'metaclusters' or 'superclusters' in rapid motion relative to each other and to the cosmological frame.

It is unlikely that this new result will dent the hot big bang picture of the

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Universe very much but our ideas about our cosmological locality are in for some revision. The Berkeley group have made an important experiment, which is clearly worth repeating. A

good test would be to do a similar series of flights in the southern hemisphere but perhaps U-2's are still not all that welcome outside US airspace. □

## Visualising jumping genes

from T. Cavalier-Smith

DNA molecules are not an inert repository of genetic information, but are frequently chopped, spliced or modified by enzymes. This occurs not only during genetic recombination, but also during replication, repair and sometimes even during nuclear differentiation; for example, extensive chopping of DNA into pieces occurs during macronucleus formation in hypotrich ciliates (Lauth *et al.* *Cell* 7, 67; 1976). Usually these changes leave the linear order of genes unaltered, but sometimes genes may 'jump' from one part of the genome to another. The first evidence for 'jumping genes' (more properly called transposable or translocatable genetic elements) came long ago from Barbara McClintock's studies of controlling elements in maize (*Proc. natn. Acad. Sci. U.S.A.* 36, 344; 1950). The study of the molecular basis of transposability is now possible as a result of the discovery over the past few years of a variety of transposable elements in *Escherichia coli*. The simplest of these are the insertion sequences (IS), short segments of DNA of defined length and sequence which can become inserted into a larger DNA molecule by a recombination process distinct from *E. coli*'s normal recombination system. They were first discovered as a result of the strongly polar mutations they cause when inserted into the *lac* and *gal* operons, but have since been found in other chro-

mosomal locations and on drug resistance (R) plasmids (for reviews see Cohen *Nature* 263, 731; 1976; Kleckner *Cell* 11, 11; 1977).

Electron microscopy of the molecular hybrids formed after denaturation and reannealing of DNA containing insertion sequences showed that there were four distinct sequences: IS1 of about 800 base pairs, and IS2, 3 and 4, each about 1,400 base pairs long. Chow (*J. molec. Biol.* 112, 611; 1977) has now used electron microscopy to throw some light on their distribution in the *E. coli* genome. Her method (much used in the past few years to study palindromes or inverted repeats in eukaryote DNA) was simply to allow the denatured DNA fragments (in length a few per cent of the total genome) to reanneal for such a short time that most of the duplexes formed were formed intramolecularly by a DNA strand folding back on itself. A single DNA strand can pair with itself in this way only if it contains at least two identical sequences that are inverted with respect to each other. If these inverted repeats are adjacent (forming a palindrome) renaturation produces a double-stranded hairpin; if they are separated by a spacer that cannot pair with itself, a double-stranded stem bearing an unpaired loop at one end is formed. Both hairpins and stem-loop structures are found in abundance in rapidly renatured eukaryote 'snap-back' DNA.

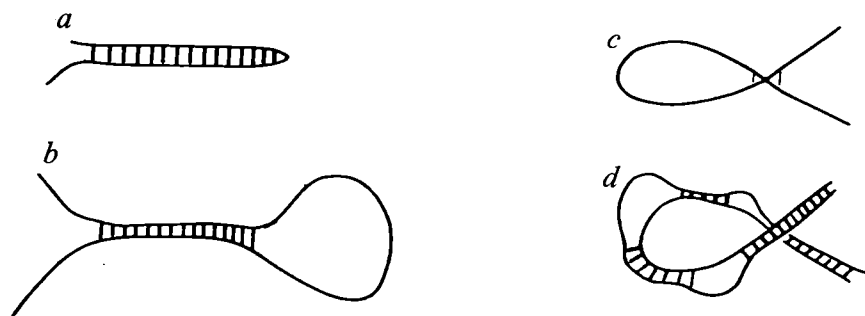
In *E. coli* DNA Chow found no hairpins (which confirms earlier work), but various stem-loop structures were

present, indicating that *E. coli* DNA does contain some inverted repeats long enough to be observed in the electron microscope (but only ones where the two sequences are separated by spacers). The duplex stems of the stem-loop structures fall into four discrete size classes, of which one corresponds to IS1 and one to IS2-4 (duplexes shorter than 400 base pairs were discounted as possible artefacts). The loop lengths are more variable but also fall into distinct classes, most being either 22,000 or 27,500 bases long, indicating a regular arrangement of the inverted sequences on the chromosome. By making various assumptions (including the identity of the 800-base-pair sequences with IS1) Chow estimates that there are a minimum of 14 IS1, and 10 IS2-4 (as well as 6 and 12 respectively of the 500-base pair and 1,000-base-pair sequences) in each genome. A similar study by Deonier and Hadley (*Nature* 264, 191; 1976) revealed inverted repeats only in the 1,300-base-pair size range, suggesting that the IS sequence content of *E. coli*'s genome differs from strain to strain.

Some of the antibiotic-resistance genes carried on R plasmids have turned out to be bounded by insertion sequences (often inverted with respect to each other). The complete genetic unit is termed a transposon and can translocate from one plasmid to another and to the bacterial chromosome without the need for a complementary sequence to be present on the host DNA molecule. It is thought that interaction between the two inverted sequences plays an important part in this translocation.

In view of the apparent importance of inverted repeats one wants to be able to identify them easily. If they are longer than about 200 base pairs electron microscopy of the snap-back DNA fraction shows hairpins or loop-stem structures, but shorter inverted repeats do not show up in this way. Much shorter inverted repeats do exist, however, for example bacteriophage Mu DNA contains a segment of DNA (the G segment—3,000 nucleotide pairs long) which is flanked by inverted repeats each of less than 50 nucleotide pairs. In the prophage (but curiously not in the infective state) the G sequence readily inverts, presumably as a result of recombination between the inverted repeats. These short inverted repeats are seen by electron microscopy after denaturation and self-annealing as a 'cross over' of the two strands at a reproducible position (as in the letter α). But in DNA less well characterised than Mu it would be hard to distinguish between such cross overs caused by limited base pairing

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The four structures visible in the electron microscope after reannealing denatured DNA containing inverted repeats. a, Hairpin; b, stem-loop structure; c, α-structure with defined crossover; d, underwound loop formed by partial pairing of two complementary α-structures.

and the chance lying of one strand on top of another normally seen in the electron microscope.

Detection of such short inverted repeats will be made much easier by the recognition by Broker *et al.* (*J. molec. Biol.* **112**, 579; 1977) that following denaturation and reannealing they give rise to a characteristic structure which they call an 'underwound loop.' When a single molecule containing two short inverted repeats anneals with itself it forms a structure with a single-stranded loop and two single-stranded tails (like an  $\alpha$ ). If the DNA is left to reanneal long enough the loops of separate molecules will come together in complementary pairs. But this pairing must be incomplete. This is because each of the loops in the two interacting molecules is topologically equivalent to a circle (because of the base pairing at the cross-over point), and two circles, unlike two linear strands, are topologically unable to undergo the high degree of winding necessary to make a complete double helix. Some pairing is possible at the expense of twisting and tangling the rest of the molecule; the 'underwound loop' that results therefore contains both double-stranded and single stretches of DNA which are clearly visible in the electron microscope. Broker *et al.* use this method to reveal the short inverted repeats at the ends of the Mu G segment (presumably the G segment is a transposon since an identical sequence is present in phage P1, where it has much longer terminal inverted repeats), those at the ends of adenovirus-2, and at the two ends of the  $\gamma\delta$  segment (a 5,700-base-pair sequence found at the boundary between F (sex factor) DNA and chromosomal DNA).

This technique will be valuable for detecting short inverted repeats separated by a spacer. I suspect that it will not be long before they are reported in eukaryote DNA. The fact that in *E. coli* the G segment can have either short terminal repeats (as in Mu) or long ones (as in P1) suggests that there is no fundamental functional difference between the long inverted repeats that abound in eukaryotes and the short ones which have so far been reported only in bacteria.

So far, ideas as to the function of palindromic sequences in eukaryotes are very vague (see *News and Views* **262**, 255; 1976). One possibility is that they have no function for the organism as a whole; they may even be disadvantageous 'selfish genes' maintained by strong positive selection at the level of the individual gene balanced by negative selection at the level of the organism; this would be entirely consistent with their tendency to trans-

## The Sun and the weather

from Roger H. Olson

OVER the past few years, research into the relationships between solar activity (such as sunspots, flares and sector boundaries) and various climate and weather parameters has become more respectable. Although the search for mechanisms has as yet yielded few results, the number of impressive statistical correlations is steadily increasing. One of the best examples is the work of J. M. Mitchell, senior climatologist with the National Oceanic and Atmospheric Administration (NOAA). Mitchell has been looking at the correlation between sunspot activity and periods of drought in the Western United States, particularly since AD 1700, established from tree ring analysis.

Mitchell has worked with C. W. Stockton and D. M. Meko of the University of Arizona Tree Ring Laboratory, in studying the size of the area covered by drought in the Western United States. First, tree ring patterns, based on several types of trees, over the past 40 years were compared with well-known periods of drought. Once this standard correlation had been established it was possible to trace periods of drought back to AD 1700. A variety of sophisticated techniques were used to compare the period and geographical extent of the drought with the sunspot cycles, which have been observed reasonably consistently back to the beginning of the period.

No evidence was found for any influence of the 11-year cycle, but there is a very strong match between periods of drought and the 22-year double sunspot cycle (the Hale magnetic cycle). The area covered by drought tended to reach a maximum about 3 years after every other minimum in the 11-year cycle, with the most recent well-documented drought being that of the mid-1950s, correlat-

ing with the sunspot minimum of 1954. (The drought of the 1970s seems to be correlating with the sunspot minimum of 1976.)

The time between droughts averaged 20.4 years, whereas the average double sunspot cycle is 22 years. The reason for this apparent discrepancy is that the best coherence between drought area and sunspots comes during times when the sunspot maxima are high. It is during such periods that the time between successive maxima and minima tends to be smaller. When the sunspot numbers are small, the period of the cycle increases, and the correlation with drought area becomes weak.

Another tree ring series, dating back to AD 1600, was made available to Mitchell by H. C. Fritts. Although this series is not amenable to the statistical treatment mentioned earlier, it does contain one additional bit of information worthy of comment. During the Maunder minimum (see *News and Views* **236**, 405; 1977), when sunspots were virtually absent from the Sun during a 70-year period, the drought periodicity persisted in the Western United States. This suggests that the visible sunspot may not be the best parameter of solar activity to be used in the study of Sun-weather relationships.

A lesson to be learnt from Mitchell's work is that different meteorological parameters may behave in dramatically different ways. His study uses the area covered by drought, rather than the amount of rainfall or some other more arbitrary parameter. Rainfall statistics are notoriously hard to work with, because of their variability and dependence on topography. □

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locate and cause mutations. A more attractive proposition is that they may be the key to understanding the mechanism of cell determination. An important feature of determination is its great stability, inheritable over many cell generations. Although in principle this is explicable in terms of an association between DNA molecules and controlling proteins (and/or RNA) which is so stable that it can persist during replication and division, I think that the idea of a semi-permanent change in the DNA itself has been unduly neglected. Holliday and Pugh (*Science* **187**, 226; 1975) have put forward a most attractive mechanism involving

methylation of short palindromic sequences. Yet another possibility would be a change in the primary sequence of the DNA mediated by insertion sequences. Such a change would be inherited automatically by daughter cells, but would in principle be perfectly reversible (for example, when the nuclei of differentiated cells are transplanted into oocytes). Such changes could be inversions, deletions, duplications or translocations and could switch genes on and off by creating or destroying the recognition sites for controlling proteins. If the IS-mediated events commonly occurred only during DNA replication and were specifically

inhibited by the incorporation of bromodeoxyuridine into DNA then a jumping gene model could explain in a unifying way all the basic features of cell determination (see Reinert & Holtzer *Cell Cycle and Cell Differentiation*, Springer, 1975), including that in the immune system, as well as the apparent inconstancy in the position of palindromes in eukaryote DNA (Perlman *et al. Cell* 8, 33; 1976). The transformed state in cancer (strongly heritable in somatic cells) could also result from a change in the primary sequence of the DNA concerned with the regulation of proliferation, as a result of stable aberrations in IS-mediated events (either spontaneous or mediated by radiation, chemicals or the insertion of viruses into the DNA).

Now that bromodeoxyuridine has been shown to inhibit bacterial sporulation (*Nature* 267, 635; 1977) it may be possible to test such ideas in more tractable prokaryotic developmental systems. There may well be fewer basic differences between prokaryote and

eukaryote genetics than has hitherto been thought. The presence of long perfect palindromes in eukaryotes in spite of their absence in prokaryotes, for example, need not be a basic difference. It could be explained simply by supposing that IS sequences and transposons are even more important in eukaryotes than in prokaryotes and are so abundant that they are often adjacent; the junction between two adjacent transposons each of which has identical inverted repeats at its termini would be a perfect palindrome. Perfect palindromes would thus be merely the coincidental result of the coexistence in the genome of large numbers of transposons with similar termini.

Although a transposon or jumping gene theory of cell determination has many attractions, the key question about DNA palindromes is: will their study provide a way towards an eventual understanding of cell determination in eukaryotes, or shall we find that they only lead us back to where we started? □

discovered a receptor for adenosine which also activates adenylate cyclase. At this stage it seems that the adenosine and  $\beta$ -adrenergic receptors compete for the same enzyme, activating it through a common GTP-binding coupling unit. The adenosine receptor, however, seems to be coupled permanently to the enzyme. Further study of this system should be very instructive in sorting out the spectrum of receptor-adenylate cyclase interactions, including the possibility raised by P. Cuatrecasas (Wellcome Research Laboratories, Triangle Park) that the receptors reside permanently in separate membrane domains from the enzyme, never coupling but rather acting through molecular messages.

As the biochemists struggle to characterise a few receptors, so the pharmacologists keep increasing the choice for future biochemists. For example,  $\beta$ -adrenergic receptors in the brain as in peripheral tissue can be subdivided into  $\beta_1$  and  $\beta_2$  types (S. Nahorski, University of Leicester) and there seem to be several distinct classes of  $\alpha$ -adrenergic receptor (D. H. Jenkinson, University College, London). Other new evidence on adrenergic receptors came in the Harden lecture delivered by S. H. Snyder (Johns Hopkins University). Both  $\alpha$  and the  $\beta$  receptors in brain, he reported, are sensitive to sodium ions. Physiological concentrations of sodium decrease the binding of agonists but increase the binding of antagonists to the receptors (and the reverse effect can be demonstrated for the angiotensin receptor). That suggests a generalisation of the two-conformation receptor model previously proposed for the opiate receptor by Snyder.

Much pharmacological evidence for multiple opiate receptors (some recently published: *Nature* 267, 577; 1977) was presented by H. Kosterlitz (University of Aberdeen). A new line of evidence comes from a comparison of the action of various opiate agonists in two strains of mice. Although enkephalin was equipotent in the two strains there was a large difference in their response to normorphine.

Most of the interest in the opiate receptor, however, remains firmly focused on the endogenous ligands. D. Smyth (National Institute for Medical Research, London) started with mention of the recently discovered very large '31K' precursor to both ACTH and  $\beta$ -lipotropin, the latter being the precursor of all the endogenous opiates except leucine enkephalin. He then summarised his (and others) evidence demonstrating high opiate-like activity of the peptide (C-fragment or  $\beta$ -endorphin) derived from residues 61 to 91 of  $\beta$ -lipotropin. C. R. Snell, from Smyth's

## Receptors and hormones

by Peter Newmark

The Eleventh Harden Conference was held on 19-23 September, 1977 at Wye College. It was organised by Dr L. Iversen.

WHEN the identity of some receptors is about as substantial as the Emperor's new clothes it is always a real pleasure to hear of the nicotinic acetylcholine receptor. Even so there is still no purified receptor to show for the huge effort that has been made in that direction along a path strewn with discarded subunits.

Everybody finds a major acetylcholine-binding subunit of around 40,000 dalton by SDS electrophoresis but there is considerable dispute about the reality and significance of other, larger subunits. Are they part of the receptor (M. Raftery, California Institute of Technology), experimental artefacts (J. P. Changeux, Pasteur Institute) or simply absent (E. Barnard, Imperial College, London)? Barnard reported one of the first purifications of the acetylcholine receptor from mammalian tissue (denervated cat hind leg muscle) and found it to be remarkably similar to the more thoroughly studied receptors of the electric organs of the electric ray and eel.

A further growing problem in the

purification of the acetylcholine receptor is that of the functional heterogeneity of its subunits: it is not known how the binding of acetylcholine is translated into ionic transport across the membrane. Is this an integral property of the receptor itself or is a separate molecular entity responsible? Substantial evidence in favour of the latter was reported by M. Eldefrawi (University of Maryland) and by Changeux. They call the molecule the Ion Conductance Modulator or the Ionophore respectively.

The holy grail of receptor purification seems to be successful reconstitution of a purified, functional receptor in a synthetic lipid bilayer. Just how elusive that grail is, even for the acetylcholine receptor, was evident from the somewhat unseemly anxiety of all those present to point out the imperfections in all reconstitution data so far published.

Also on the slow but steady path to purification is the  $\beta$ -adrenergic receptor (A. Levitzki, Hebrew University of Jerusalem). At the same time Levitzki's group have accumulated kinetic evidence that the  $\beta$ -adrenergic receptor is not permanently coupled to adenylate cyclase. Instead a 'collision coupling' mechanism is involved in activation of the enzyme following ligand binding. That evidence comes from work on the turkey erythrocyte in which Levitzki has very recently

laboratory, next presented evidence for the existence of extracellular endopeptidases that could degrade  $\beta$ -endorphin to  $\gamma$ - and  $\alpha$ -endorphins and to methionine enkephalin. He also showed that various experimental conditions for the extraction of endogenous brain opiates could favour activation of the degradative endopeptidases. Any suggestion however that such degradation accounted for the isolation of enkephalins rather than larger peptides from the brain was quickly discounted by Kosterlitz who pointed out that enkephalins have been extracted as the major brain opiates in conditions in which  $\beta$ -endorphin is not degraded.

Confirmation of that came from R. Miller (Wellcome Research Laboratories, Triangle Park) who then posed the question of the origin of brain enkephalin. The small amounts of  $\beta$ -lipotropin detected in the brain (Snell) indicate that enkephalin is metabolically derived from precursors synthesised *in situ*. Less likely is that brain enkephalin has its origins in  $\beta$ -endorphin released from the pituitary, especially since Miller showed that hypophysectomy had almost no effect on brain enkephalin content.

What then is the fate (purpose?) of the pituitary endorphin? The discovery of a hand-in-hand secretion of  $\beta$ -endorphin and ACTH into the bloodstream from the pituitary (*Science* 197, 1367; 1977) at least opens the possibility that it plays some part in the physiological response to stress.

Another group of peptide hormones to receive attention was the insulin family. T. Blundell (Birkbeck College, London) explained how it was possible to predict the basic three-dimensional structure of insulin growth factor (previously called non-suppressible insulin-like activity) and relaxin from their recently completed amino acid sequences and knowledge of insulin and proinsulin three-dimensional structure. When the structures are compared with that of insulin it becomes clear why insulin growth factor has insulin-like activity but relaxin has not.

Many of the remaining peptide hormones were encompassed in one grand scheme by L. Kohn (National Institutes of Health, Bethesda) who reviewed his evidence that the glycoprotein hormones are composed of two molecular subunits as is cholera toxin. One subunit is responsible for an initial interaction with a hormone-specific membrane ganglioside on the surface of target cells. As a consequence, the other subunit (characterised by a sequence homology that is present also in oxytocin and vasopressin) can penetrate the membrane, after which adenylate cyclase is activated. Recent evidence favours the

inclusion of tetanus toxin and interferon under the same mechanistic umbrella.

Kohn also reported evidence that one of the glycoprotein hormones, thyrotropin, causes an alteration in electrical potential across the membrane of cultured thyroid cells before stimulating adenylate cyclase (*Proc. natn. Acad. Sci. U.S.A.* 74, 2352; 1977). That led him to speculate that ion flow may be the primary event for all the ligands under his umbrella. Primary events were also the concern of R. Michell (University of Birmingham) who summarised the evidence that favours a crucial role for increased phosphatidylinositol turnover following ligand-receptor interaction in many non-cyclic AMP-dependent systems. Unfortunately the evidence, although substantial, is indirect and badly in need of direct, critical confirmation.

Finally a word in praise of the immunologists. They (M. Raff, University College, London; F. Melchers, Basel Institute for Immunology; H. Wigzell, University of Uppsala) superbly met the challenge of presenting their field to a largely ignorant audience in the space of an evening. □

## Colonisation of a volcano inside a volcano

from Jared M. Diamond

THE regrowth of life on islands sterilised by volcanic explosions has offered biologists 'natural experiments' for studying colonisation. Classics of this type include studies of Krakatau (in Indonesia, exploded in 1883; Docters van Leeuwen *Ann. Jard. Botan. Buitenzorg*, 56-57, 1; 1936; Dammerman, *Verh. K. ned. Akad. Wet. Afd. Natuurk.* II, 44, 1; 1948; Borssum Waalkes, *Ann. Bogorienses*, 4, 5; 1960) and Surtsey (off Iceland, arose from the sea in 1963; Fridriksson, *Surtsey*, Wiley, 1975). From observations of these islands, biologists have sought answers to questions such as: How do species differ in their ability to reach an empty island? Of the arriving species, who survives, and why?

A promising new study, by Eldon Ball (Australian National University), Joe Glucksman (Department of Primary Industry, Papua New Guinea), and Jean-Marie Bassot (CNRS, France) is of Long Island off the coast of New

Guinea. A recent visit adds to information that Ball and his coworkers gathered on five previous visits to Long since 1969 (Bassot & Ball *Papua New Guinea Sci. Soc. Proc.* 23, 26; 1972; Ball & Glucksman *Proc. R. Soc. Lond. B* 190, 421; 1975; Ball & Johnson in *Volcanism in Australia*, 133-147 (ed. R. W. Johnson), Elsevier, 1976; Ball *Australian Nat. Hist.* 19, 12; 1977). Factors making Long especially interesting are that its tropical location exposes it to a rich pool of colonists, that it is not just a single island but a nested set all of which have been at least partially defaunated in the past 300 years, that Ball's teams are virtually the only humans to have reached the innermost island (reducing the risk of species introductions by humans), and that there have been multiple colonisation episodes of this inner island associated with successive eruptions.

Long Island lies in the Bismarck Sea 30 miles northeast of New Guinea. It is a forested island of 170 square miles, in the centre of which is a crater lake (Lake Wisdom) of 37 square miles, in the centre of which is a small active volcano (Motmot Island) of about 10 acres, in the centre of which was (from 1968 to 1973) a small crater lake of about 1 acre. By a combination of biological, geological, historical and anthropological detective work, Ball has amassed evidence that an 18th-century explosion buried Long's former biota under tens of metres of lava. Lake Wisdom probably dates from that explosion, at least in its present form. The inner island, Motmot, is the tip of a submerged cone that erupted to form an island in 1943, was twice eroded down to the lake level and recreated by eruptions, and has existed continuously since 1968.

The outer island, Long itself, is already covered with rainforest. Its tree species are ones whose seeds arrived either by floating in the sea (for example *Terminalia catappa*, *Barringtonia* sp.), or else in the guts of birds (*Ficus*, *Canarium*, and *Eugenia* species, probably brought by fruit pigeons). In these forests now live nearly a full quota of bird species predicted for an island of Long's area (except for a deficit of montane bird species), as well as lizards (including the large *Varanus indicus*), snakes, bats, and the arboreal marsupial *Phalanger orientalis* (probably introduced by the humans who recolonised Long in the 19th century).

Lake Wisdom is remarkable for its depth of 1,200 foot, and for having high oxygen concentrations and aerobic invertebrate life down to the bottom. Most other large lakes in this region have anoxic bottoms lacking a bottom fauna. Two factors that may transport

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oxygen to the bottom of Lake Wisdom are wind circulation and convection currents caused by heating of the water around Motmot volcano. Although the lake's surface is at an elevation of 620 foot and the bottom is thus well below sea level, the water is nevertheless fresh down to the bottom, implying that the lake basin is sealed.

Lake Wisdom's biota is impoverished, compared with that of an older 'control' lake nearby (Lake Dakataua), probably because the lake is little more than two centuries old. The most striking absences are of vascular aquatic plants, copepods, fish, and turtles. However, there are many species of benthic algae, invertebrates (aquatic insects, molluscs, crustaceans, and one species of sponge), and one species each of nesting duck and grebe. Winds and birds probably brought the plants and invertebrates. But the lake also contains at least one adult crocodile. Crocodiles periodically reach the sea-coast of Long in floating rafts of vegetation from New Guinea. How they got several miles inland and up and over the steep crater rim into the lake remains a mystery.

The inner island, Motmot, is difficult of human access because of the steep crater rim, lack of human population on Lake Wisdom's shore, the four miles' distance over the wind-tossed lake from the shore, crocodiles in the lake, and near-boiling water temperatures on some sides. Ball and his co-workers have variously reached Motmot from the lake shore by native-built outrigger canoe, collapsible aluminium boat, dinghy, or swimming while pushing a raft and avoiding the areas of near-boiling water. On Ball's first visit in 1969, 1 year after recreation of Motmot by an eruption, the sole mature plant was a sedge, the sole land invertebrate a lycosid spider. Over the next 4 years the number of plant and invertebrate species respectively increased to 17 and 20, until a new eruption in 1973 destroyed the inner crater lake and wiped out most plants except a sedge. Ducks and swallows nest on Motmot, migrant shorebirds from the northern hemisphere periodically visit, and a falcon visits from Long itself to devour its prey of small birds.

How do the plant and invertebrate colonists reach Motmot? Some arrive wind-blown by aerial rafting. Many are washed up on Motmot's beach, having floated from Long across Lake Wisdom by themselves or on floating logs. Ball found five species of invertebrates on a log that had floated past Motmot. Numerous seeds that wash onto the beach sprout but fail to survive the combination of heavy wave erosion and changing water levels. Some seeds come attached to the feathers and feet of the

nesting ducks and grow mainly in the areas that the ducks frequent. Still other seeds arrive in the guts of the falcon's bird prey.

What invertebrates colonise Motmot, where do they live, and what do they eat? On the beach are beetles, earwigs, ants, and bugs, living off washed-up organic material. Under an algal crust on the inner crater lake are collembolans and rove beetles. Around this lake are ants, scavenging or eating smaller insects. It was a surprise to find a lycosid spider as the earliest land colonist, since they are strict carnivores and since scavengers dominated the early colonists of Krakatau and Surtsey (but a spider was the first organism found on Krakatau after it exploded). What would a solitary carnivore eat? Probably aerial plankton; but lycosids can gorge themselves and survive half-a-year without food.

As yet, virtually no attention has been paid to comparing the species composition of Long's flora and insect fauna with the source biota of New Guinea and New Britain. This would be a rich data base for understanding who does and does not colonise. It could also suggest how much time is necessary for genetic divergence and subspecies formation. For example, the description of an endemic bird subspecies from Long (Salomonsen *Breviora Mus. Comp. Zool. Harvard*, no. 254; 1966) suggests formation of a bird subspecies within two centuries. □

## Tibetan meteorology

from T. B. Tang

IN its working conference held in June, the Academia Sinica confirmed that weather and long-term climatic forecasting has been selected as a key research topic in China; in the same month, its Institute of Atmospheric Physics launched a new journal—*Atmospheric Science*—devoted principally to climatic research. (From the second issue onwards this journal is available and has a table of contents in English). Meteorologically, the Chinghai-Tibet Plateau in western China is of immediate relevance, if only because of its vastness (one-fourth of China's land area). The Plateau has immense dynamic effects on the atmospheric circulation in the Northern Hemisphere and constitutes an important influence on the weather and climate of areas eastward as far as the Japanese Islands.

Recently, there has been much

scientific activity in Tibet. For instance, exploiting the high altitude, the Chinese have set up there an observational station for cosmic rays. Hard on the heels of the completion of field work for the comprehensive geological and biological survey (see *News and Views* 269, 12; 1977), the first major meteorological programme commenced in July this year and, after three months, has now come to a close (*Hsinhua News*, 10 October).

The research programme dealt with weather patterns on the Plateau in the summer, and was carried out collectively by personnel of the Central Meteorological Bureau, of provincial bureaux in northwest and southwest China and the Army, as well as by local meteorologists.

The work included the study of meteorological data collected between 1969 and 1976, the analysis of cloud photographs taken by satellites in recent years, and field surveys along the middle reaches of Yalu-tsaupo River. The detailed findings are not yet available. However, the general picture obtained is that in summer, a high pressure air mass forms in the upper level of the atmosphere; when it moves eastward, it causes drought in the other parts of China. At low levels in the atmosphere there also frequently occur low-pressure eddies and shear lines. These are the weather systems responsible for precipitation in summer on the plateau, bringing damaging heavy rain. In certain circumstances they move eastward and cause devastating floods in southwest, central, and eastern inland China. An understanding of the formation, evolution, and distribution of these eddies and shear lines, as well as their directions of movement and seasonal variations, and the relationship between these factors and precipitation is, therefore, vitally important to weather forecasting and the mitigation of natural calamities.

In the present connection it is of interest to note a review article discussing climatic changes in the Plateau for the past 500 years, which appeared in the September 1977 issue of the popular science journal *Ke Xue Shi Yan*. The article reported that an

### Correction

In the article 'Carbon fixation pathways' (*News and Views* 269, 201; 1977) paragraph 5, line 7 and following should read "Measurements of leaves of quantum yield for CO<sub>2</sub> uptake show the following. At normal oxygen tension and 30 °C, C<sub>3</sub> and C<sub>4</sub> plants show similar efficiencies, but as CO<sub>2</sub> tension increases the efficiency of C<sub>3</sub> metabolism increases whereas C<sub>4</sub> remains constant."

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annual mean temperature profile has been worked out, using evidence from the analysis of the annual growth rings of trees; samples were selected from trees found at around 4,300 m above sea level. On the other hand, trees growing at about 3,000 m on forest edges, where moisture rather than warmth becomes growth-limiting were used for estimating annual precipitation. In spite of greater variations from place to place, the picture has emerged that in the 1770s, the 1830s and the early 20th century, annual precipitation was on the whole higher than that at present. It was lower, however, during the longer periods of the late 18th century, the second half of the 19th century, and the mid-20th century. In the past 20 years rainfall has been steadily decreasing by a relatively large total amplitude of approximately 10%. It has been possible to check this picture independently with the observed history of water levels in lakes (from field surveys or satellite pictures), the motion of glaciers (especially their regression), the movements of snow-lines as well as water table levels, and in a few cases with documented records. In general, the results are all consistent with the proposed former variations in annual mean temperature and precipitation. □

## Singlet oxygen in chemistry and biology

from R. B. Cundall

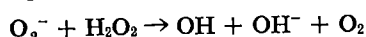
An International Conference on Singlet Oxygen and Related Species in Chemistry and Biology was held on 21–26 August, 1977 at the Whiteshell Nuclear Research Establishment in Pinawa, Manitoba. Many of the papers will be published in a special issue of *Photochemistry and Photobiology*.

THE Earth has an atmosphere containing diatomic triplet oxygen ( $^3\Sigma_g^-$ ); essential for life in the form in which we know it. Consequently an understanding of the chemistry of all oxidation processes which have both beneficial and harmful effects is one of the major problems confronting both biochemists and chemists. The meeting aimed to foster the interaction of physical chemistry with biology in elucidating oxidative transformations, and attracted scientists from fields as diverse as medicine and molecular physics.

A feature of oxygen chemistry is

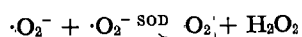
the interconvertibility of reactive oxidising transients. Attention was mainly directed to the behaviour of  $^1\Delta_g\text{O}_2$ ,  $^1\Sigma_g^+\text{O}_2$ ,  $\cdot\text{OH}$ ,  $\cdot\text{O}_2^-/\text{HO}_2\cdot$ ,  $\text{O}_3$ ,  $\text{H}_2\text{O}_2$ , organic peroxides and hydroperoxides, and derived species such as some of the oxidising inorganic radical anions. Different groups of specialists, showing common interests in oxidation mechanisms presented new work using a wide range of techniques. Particularly prominent were laser photolysis, pulse radiolysis, luminescence, chemiluminescence, ESR, NMR, as well as conventional biochemical techniques, steady state photolysis and radiolysis with product analysis.

Quantitative measurements, mainly by pulse radiolysis, show hydroxyl radicals to be very reactive and to interact unselectively with most biological molecules at rates which are practically diffusion controlled. The fate of the secondary radicals formed by attack on substrates was very much a subject for discussion since it is clear that oxidation in biochemical systems must generate a species of reactivity comparable to OH. The process usually cited to explain this has been



a component of the now classical Haber–Weiss mechanism (1934). This cannot be the reaction involved since it has been established as slow (B. H. J. Bielski, Brookhaven National Laboratory; G. Czapski, Hebrew University, Jerusalem). Alternatives proposed were metal-mediated processes, enzyme-metal catalysis or involvement of peroxides, particularly those derived from lipids.

The low reactivity of the  $\cdot\text{O}_2^-/\text{HO}_2\cdot$  system is now widely agreed and it is clear that in general  $\cdot\text{O}_2^-$ , like  $\text{H}_2\text{O}_2$ , reacts only with such readily reducible molecules as those containing labile SH groups. Nevertheless the biochemical role and significance of the superoxide anion is puzzling. The xanthine oxidase enzyme system is an efficient  $\text{O}_2^-$  generator. The superoxide dismutase (SOD) whose function was first defined by one of the participants, I. Fridovich (Duke University, North Carolina), apparently exists solely to bring about



The nature of this type of enzyme and its properties was a dominant topic in both presentations and discussion. The pulse radiolysis technique has been particularly successful in elucidating the mechanism of these metallo-enzymes (E. M. Fielden, Institute of Cancer Research, Sutton, Surrey). The

use of catalase to remove  $\text{H}_2\text{O}_2$  completes an enzyme trio used to a large extent in mechanistic studies.

Radiation chemistry now has an established role as the most prolific source of information on free radical reactions in aqueous solution and several papers gave evidence of progress in the area. A significant contribution to radiobiology was the demonstration that SOD is an effective radioprotector exerting a protective role even when administered after radiation doses have been delivered (A. Petkau, Whiteshell Nuclear Research Establishment). This clearly shows where knowledge of primary radiation effects and oxidative enzyme reactions can be combined to advantage.

The role of  $^1\text{O}_2$  in biochemical systems is not yet clear. It does not seem to be formed in significant yields from  $\text{O}_2^-$ , in polar systems at least, although it can be formed by chemical reactions which generate it directly or by energy transfer to  $^3\Sigma_g^-\text{O}_2$  from a product formed in the triplet state. The paper of J. Cadet (Centre d'Etudes Nucleaire de Grenoble) in which methodical attempts to generate separately  $^1\Delta_g\text{O}_2$  as well as OH and  $\text{O}_2^-$  and then examine their reaction with DNA constituents shows how the subject is likely to progress in the immediate future.

The use of dye photosensitisers in research on the so-called photodynamic effect is an area in which an important role for  $^1\Delta_g\text{O}_2$  is beyond dispute. The complications arising in particular cases from the simultaneous participation of the Type I (free radical) and Type II ( $^1\Delta_g$ ) processes were described by several authors and the behaviour related to dye and binding properties (T. Ito, University of Tokyo). A number of papers dealt with experimental techniques, especially interesting were those on the use of dye lasers for excitation of singlet oxygen.

Many interesting studies on specific systems were reported; for example M. Kasha (Florida State University) has found that pressurisation by oxygen produces complexes which remain even when the pressure is reduced. The  $^3\text{E}_{1u}$  state of benzene has been revealed and the  $\text{T}_1 \rightarrow \text{S}_0$  emission from pyridine seen for the first time by this method.

An experiment in conference organisation was a public meeting held on the first evening in which five speakers gave short presentations of interest relevant to the content of the scientific programme to a wide audience. Topics were skin sensitivity to light (M. M. Mathews-Roth, Harvard Medical School), free radicals and ageing (J. D. Crapo, Duke University, North Carolina), sunlight and air pollution (B. D. Goldstein, New York University), the ozone layer (H. R. Rawls,

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Louisiana State University), and the fate of spilled oil (R. A. Larson, Stroud Water Research Centre, Avondale, Pa.). More than 300 people from a small community attended and there was active questioning and discussion. This might be a possible way for scientists to bring their scientific work and opinions directly, rather than through the media, to a lay audience, especially on controversial topics. □

## Thin film technology

from C. W. Pitt

The first of an intended series of international conferences on ion-assisted thin film coating techniques was held in Edinburgh on 8–10 June 1977. The Proceedings are available from CEP Consultants, Edinburgh. The next IPAT conference will be held in London in July 1979.

THE presentations covered a wide range of the experimental and production processes, ranging from ion plating, ionised clusterbeam technology, r.f. sputtering, magnetron sputtering, sputter-ion plating, ion implantation and activated reactive evaporation and plasma deposition. An equally large range of applications of the various methods was presented including tribology, tool coatings, corrosion resistant turbine and airframe components, decorative finishes, gas storage, semiconductor, optical and solar devices, electronic components and steel hardening.

A number of the papers were of particular interest to electrical and electronic engineers and solid-state physicists. The paper by T. Takagi *et al.* (Kyoto University) on ionised cluster-beam technology suggested that the required incoming material mobility on the substrate can be achieved by partially ionising clusters of atoms from an adiabatic expansion source and, provided that the deposition rate is maintained at least  $10^3$  higher than the residual gas impingement rate, single crystal and near bulk density films of a wide range of materials may be deposited. The technique has been applied in numerous ways—for example, epitaxial silicon on silicon, InSb on sapphire, GaAs on Cr: GaAs. Silicon solar cells grown n on p, have been fabricated with exposed-surface-layers of 140 Å and which exhibit 70% of the peak output voltage from 450 nm to 950 nm. Low resistance noble metal films deposited on glass substrates have

demonstrated near bulk resistivity in films 200 Å thick; thicker films of the same metals have been used as interconnections on passivated semiconductor chips and have successfully covered 8 µm oxide steps. Furthermore, ohmic contacts have been made to semiconducting material (Ag on n-type silicon, AuBe on p-type GaP) without interface metals or sintering. Ionised cluster-beam deposition on Mn-doped ZnS was also reported to have produced d.c. electroluminescent displays of similar properties to those produced by evaporation and subsequent implantation of the dopant. The superior adhesion properties of the process were demonstrated by the fabrication of flexible printed circuit board which meets the requirements of IPC-FC-240B specification.

K. Jones *et al.* (ICI, Runcorn) have also examined Au, Cu, and Al layers deposited on several types of flexible plastic substrates—polyethersulphone, polypropylene, polyimide and polyester. They concluded that the Cu: polyethersulphone system was particularly suited for flexible pcb construction by the ion-plating process. Ion plating produced good decorative finishes for most of the metal/plastic combination. Sims, in a review of ion-plating applications, again mentioned the fabrication of printed circuit board material. He also cited a number of less obvious applications such as battery-grid coatings which result in an improvement in the energy storage efficiency of lead/acid cells, aluminium coatings for steel and plastic lamp-reflectors and which are subsequently protected by ion-plated glassy layers, and also coatings for X-ray anodes for use in the soft wavelength spectrum. B. Heinz (Leybold Heraeus, Hanau) reported the use of electron-beam ion plating for forming the contact layers of noble metals on reed-relay blades. He and his colleagues have found that the switching lifetime of the plated blades could be enhanced by a factor of 10 times in appropriate conditions, and that the process produced blades with reduced sticking probability and contact resistance. The process is in industrial production.

Anti-reflective and protective coatings for germanium lenses for infrared imaging were discussed by E. Henderson (Pilkington, St Asaph). Although the experiments reported were in the early stages, it was clear that serious consideration is being given to the use of ion plating for depositing ZnS anti-reflection coatings on five-element large-area zoom lenses for operation at 8–12 µm in corrosive atmospheric conditions, in aircraft (for example). Preliminary data indicate an improvement in lifetime of four to eight times in

adverse conditions. C. W. Pitt (University College, London) reviewed the r.f. sputtering process, with particular emphasis on the capability of this process for depositing dielectric films with good optical properties. The technique has been found to be attractive for fabricating optical waveguiding components for integrated-optics applications. Hard graphitic carbon deposits may be prepared by vapour deposition from a hydrocarbon gas excited into a plasma-phase by an r.f. field S. M. Ojha (University of Sussex) presented some details of this process and of the films produced—it was projected that the rather impressive hardness (unscratched by tungsten carbide) and chemical passivity (insoluble in any of the solvents and acids used, including hydrofluoric acid) might lead to applications in semiconductor passivation and infrared filters.

The increasing use of nuclear power stations for generating electricity has produced several contentious issues, not least of which is the disposal of radioactive wastes. The storage of active gases is particularly sensitive in view of the rapid dispersal if the container is damaged. D. S. Whitmell *et al.* (UK Atomic Energy Authority) have devised an ingenious method of containing radioactive <sup>85</sup>Krypton for the required 100–200 yr and which greatly reduces the potential danger if the container is damaged. The gas is incorporated into a copper thin film matrix by plating a thin film of the metal on a substrate by d.c. sputtering, followed by a reversal of the field so that gas ions are bombarded at the deposited film, penetrate and are trapped. The process is then repeated many times until a thick deposited layer with occluded gas entrapment is built up. It was claimed that up to 5% (atomic) gas occlusion could be achieved—a similar storage capability to a high pressure gas cylinder (approximately 170 l of gas at NTP per l of metal). Damage to the deposit results in very little gas release and only from the area local to the damage.

Two papers on ion implantation, by C. Dearnley *et al.* (Atomic Energy Research Establishment, Harwell) and by V. Ashworth *et al.* (University of Manchester Institute of Science and Technology) suggest that semiconductor devices are not the only area in which the electronic/electrical industry will be influenced by this process. The papers primarily dealt with ion implantation of dopants into metals as a means of altering the wear-resistance of the host metal. But it was apparent from the results of tests on the electrochemical potentials of the doped materials that cathodic protection with improved efficiency may well be feasible. □

# review article

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## Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments

W. Charles Evans\*

*Methods of aerobic degradation of aromatic compounds in the biosphere are well understood, but it is only relatively recently that it has been shown how some bacteria can also degrade these substrates in the absence of molecular oxygen. This occurs by photometabolism (*Athiorhodaceae*), nitrate respiration (*Pseudomonas* and *Moraxella* sp.) and methanogenic fermentation (a consortium) in which the benzene nucleus is first reduced and then cleaved by hydrolysis to yield aliphatic acids for cell growth. These methods may be used by microbial communities to catabolise man-made pollutants.*

THE biogeochemical cycles which operate on Earth are essential for the maintenance and continuation of life as we know it. Solar radiation provides the energy which is harnessed by photosynthesis. Relatively simple inorganic chemicals (supplied by soil minerals, natural waters and air) are utilised by autotrophs for their biochemical processes; these feed the heterotrophs. After death, the constituents of living organisms are mineralised again by microorganisms. This dynamic sequence of events illustrated by the biological carbon cycle (Fig. 1) has evolved over a geological time scale and in a variety of physical conditions.

Although there is diversity and increased biochemical complexity as we pass from bacteria to plants and animals, their cellular metabolism and the structures concerned with the basic reactions of life have much in common. Yet, their biosynthetic and degradative powers differ markedly. Plants are unique in making large quantities of insoluble polymers, including the aromatic macromolecules—lignins and tannins—which only microorganisms can degrade. This sector of the C cycle shown in Fig. 2 has the slowest turnover rate of all natural products<sup>1</sup>; soil humus, however, confers beneficial properties on this medium for plant growth.

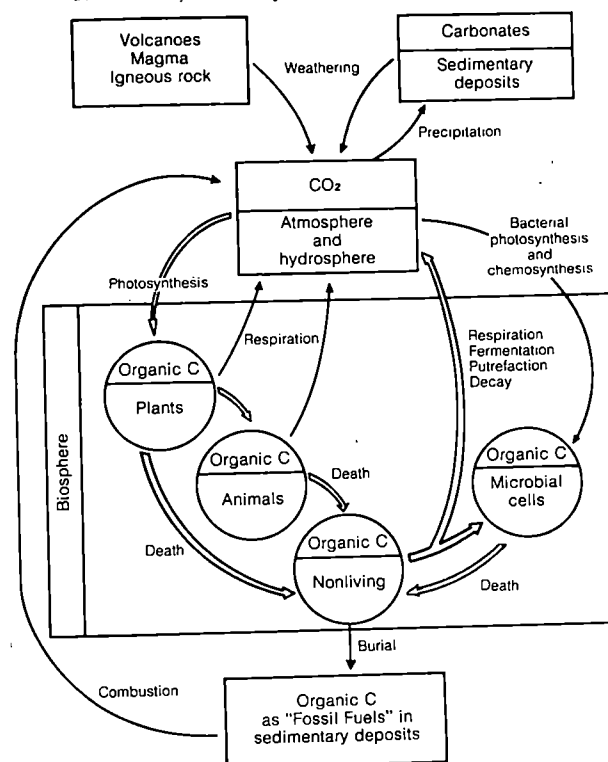
Microorganisms have evolved enzymes of impressive versatility to metabolise natural products; use is made of these in the food industries, water purification and sewage treatment. Science is also applied to control other sectors of the biological C cycle, for example, artificial fertilisers, herbicides, fungicides and insecticides to increase food production; detergents and pharmaceuticals for health reasons. Many of these are synthetic, xenobiotic, aromatic organic chemicals and together with the ever increasing waste products of industry, present an added burden for the mixed microbial populations of soils and natural waters to dissimilate.

Three general methods exist for the catabolism of organic compounds. (1) In aerobic respiration, the oxidation stages occur at the expense of molecular oxygen as the terminal electron acceptor;  $O_2$  is also a reactant for oxygenase enzymes since it is incorporated into the products. The availability of  $O_2$  is often a limiting factor in mineralisation. (2) In anaerobic respiration, bacteria use inorganic electron acceptors to metabolise substrates in the absence of air. Thus,  $NO_3^-$  is reduced to  $N_2$  or  $NH_3$ ;  $SO_4^{2-}$  to  $S^{2-}$ ; and  $CO_2$  to methane ( $CH_4$ ). Although pathways of degradation are usually identical in aerobic and anaerobic respiration, this is not the case for aromatic compounds. (3) In

fermentation, no external electron acceptor is required, the carbon source is degraded anaerobically by a series of reactions that release energy by substrate-level phosphorylation. There is a wide range of fermentation products; aerobic processes are necessary to complete that part of the carbon cycle initiated this way.

Clearly, the utilisation of any of these pathways is determined by the nature of the habitat. Aerobic and anaerobic environments exist in the biosphere; if the oceans are taken into consideration, the size of the latter far exceeds that of the former<sup>2</sup>. Conditions must also be such as to sustain microbial life—nutrients must be available and the pH and temperature favourable—for mineralisation to occur. Vast quantities of organic matter was seques-

Fig. 1 The biological carbon cycle. (Modified from original in *Introduction to Bacteria and their Ecology*, by R. N. Doetsch & T. M. Cook, University Park Press, Baltimore, 1973).



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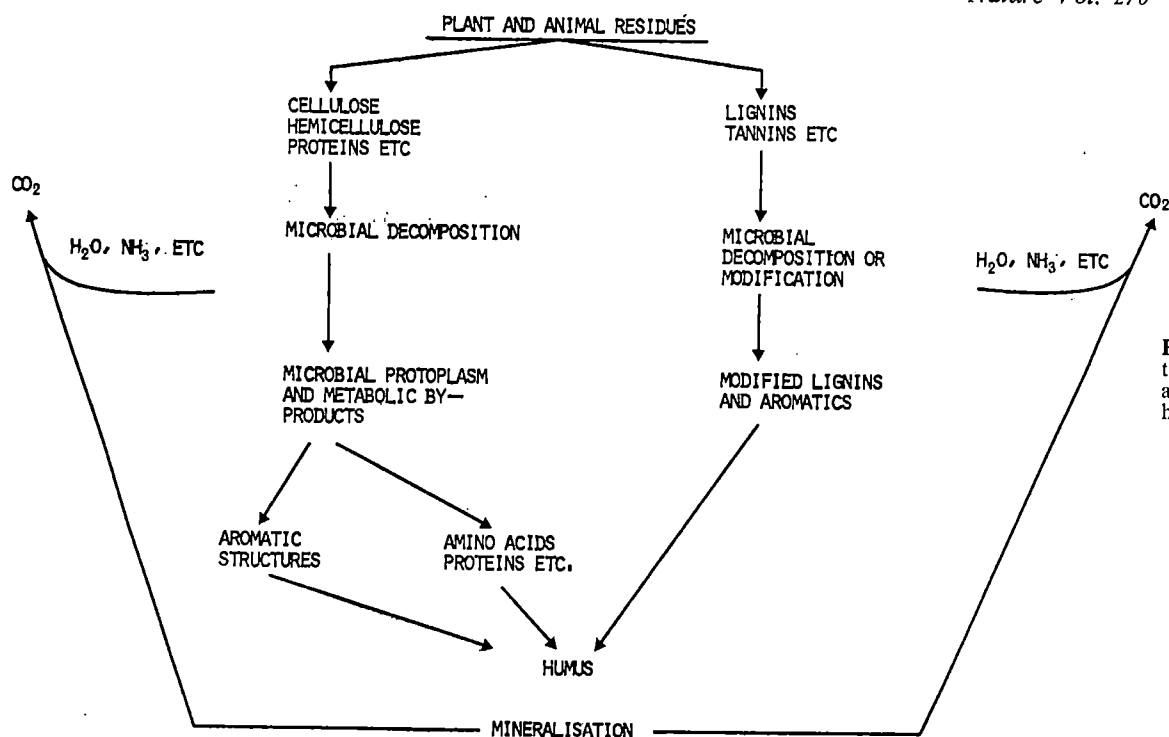


Fig. 2 Organic matter decomposition and formation of humic substances in soils.

trated in the lithosphere in ancient times—now forming the coal, oil and natural gas measures—which remained virtually intact until man began to bring these sources of carbon and energy into circulation again. Soils and sediments provide all the conditions mentioned above; the continuous alternation between aerobic and anaerobic habitats control the biological carbon, nitrogen, sulphur and other cycles in soils<sup>3</sup>.

These factors are well illustrated when we consider the various methods of microbial degradation of aromatic compounds in nature; they also have a basic relevance to the problems of environmental pollution.

### Catabolism of aromatic compounds

It is about 30 years since the salient chemical features of the aerobic *ortho* (intradiol) pathway of bacterial aromatic-ring metabolism was elucidated<sup>4-8</sup>. This was followed by the discovery of the alternative *meta* (extradiol) method by which some bacteria cleave the benzene nucleus<sup>9-12</sup>. These aerobic pathways are initiated by microbial mono- and di-oxygenases; molecular oxygen is essential for them to function since it is incorporated into the reaction-products<sup>13</sup>. They have been extensively reviewed<sup>14-16</sup> and need no repetition here.

Natural selection among microorganisms has explored the chemical possibilities of how to convert stable aromatic structures into useful metabolites; all aerobes employ the device of oxygenative ring-cleavage. In anaerobic conditions this is prohibited; evolutionary considerations indicate that the only remaining options available to bacteria in the primitive biosphere with its anoxic atmosphere are either hydration or hydrogenation followed by non-oxidative ring fission. Chemical considerations indicate that for additions to an aromatic nucleus to occur, the system of bonding characterised by extensive delocalisation of the  $\pi$  electrons must be converted into one in which little of the delocalisation energy remains. Hydrogen saturates the aromatic ring under moderate conditions, whereas addition of water is not observed and is thermodynamically improbable. Predictions made on chemical reactivity alone are not necessarily valid for enzyme-catalysed reactions; indeed, both ideas were at one time entertained. It has now been established that in all cases investigated where aromatic substrates are degraded under anaerobic conditions, the microorganisms utilise the remarkable biochemical device of initial ring reduction, first announced by Dutton and Evans<sup>17</sup>.

Indications that anaerobic methods of aromatic-ring met-

abolism exist in nature which permit cleavage without the participation of oxygen *per se* was provided by Tarvin and Buswell<sup>18</sup>. They reported that benzoate, phenylacetate, phenylpropionate and cinnamate were completely utilised with the production of CO<sub>2</sub> and methane by a sewage-sludge inoculum in strictly anaerobic conditions. During the past decade advances have been made in understanding the biochemistry of this phenomenon. It transpires that the anaerobic dissimilation of the benzene nucleus occurs in at least three different sets of biological conditions and it is the main purpose of this article to delineate progress made in each case.

### Anaerobic photometabolism of benzoate by the Athiorhodaceae

Several species of the purple non-sulphur bacteria, the *Rhodospirillaceae* are able to grow at the expense of simple aromatic compounds as sole carbon source both anaerobically in the light by photosynthetic means and aerobically in the dark by respiration. Proctor and Scher<sup>19</sup>, studying the photometabolism of benzoate by *Rhodopseudomonas palustris*, assumed that the 'bound oxygen' produced by the light reaction (van Niel<sup>20</sup>) was equivalent to molecular oxygen; a pathway similar to the known aerobic methods of dissimilation was therefore suggested. Leadbetter and Hawk<sup>21</sup>, and Dutton and Evans<sup>22</sup>, however, obtained results which made this hypothesis untenable; *Rhodopseudomonas palustris* cells grown photosynthetically on benzoate or the hydroxybenzoates showed no respiratory activity with these substrates in aerobic conditions. Furthermore, such cells were devoid of the enzymes of the aerobic pathways, and the familiar intermediates were not detectable in culture. Figure 3 illustrates dramatically the inhibitory effect of oxygen and the obligatory requirement for light in the photometabolism of benzoate.

It became apparent that another method of biochemical attack on the benzene nucleus must occur in these conditions; the addition of hydrogen seemed to us a distinct possibility. Unlabelled 'test' intermediates to be expected if a reductive pathway operated, were incubated with cell suspensions of *Rhodopseudomonas palustris* actively photometabolising (U-<sup>14</sup>C) benzoate (I); after appropriate time intervals the test compounds were extracted and examined for any isotope exchange. Cyclohexanecarboxylate (II), cyclohex-1-enecarboxylate (III), 2-hydroxycyclohexanecarboxylate (IV), 2-oxocyclohexanecarboxylate (V), and pimelate (VI) became labelled—indicating that the seven carbon atoms of benzoate remain together until after the

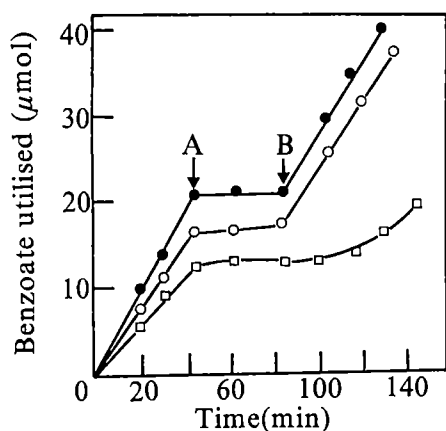


Fig. 3 The effects of air, light and darkness on the photometabolism of Benzoate by *Rhodopseudomonas palustris* cells. The initial conditions were anaerobic with illumination. In the period (A-B) between the arrows the conditions were: air-light (○), air-dark (●) and anaerobic-dark (□).

ring cleavage stage. These results suggested<sup>17,23</sup> a series of reactions involving the reduction of benzoate (or a derivative) to a cyclohexanecarboxylate moiety followed by a coenzyme-A mediated  $\beta$ -oxidation sequence. Because the reduced acid is alicyclic, instead of the usual release of acetyl-CoA, breakage of the bond in the 1,2 position occurs to yield pimelate (or its equivalent). This new reductive pathway illustrated in Fig. 4 subsequently received independent support from Guyer and Hegeman<sup>24</sup> who showed that the behaviour of *Rhodopseudomonas palustris* mutants was consistent with such a scheme. We have recently demonstrated the occurrence of these reactions using sub-cellular fractions from *Rhodopseudomonas palustris* grown photosynthetically on benzoate with the following results (ref. 25 and D. O. Lunt and W.C.E., unpublished). (I) A washed chromatophore suspension reduced benzoylphosphate but not benzoate or benzoyl-CoA; light and anaerobic conditions were essential for this to occur. A cell-free extract in the presence of CoA, ATP,  $\text{NAD}^+$  and  $\text{Mg}^{2+}$  ions converted cyclohex-1-enecarboxylate to pimelate in anaerobic

or aerobic conditions and in the light or dark.

These results provide strong evidence for the presence of a light-dependent membrane bound proton-translocating redox system in these chromatophores; the low potential reductant may be a ferredoxin, which also plays a part in photosynthetic electron transport. In addition, they confirm the presence of the appropriate  $\beta$ -oxidation suite of enzymes in these cells responsible for the subsequent series of reactions resulting in ring-cleavage.

Although all members of the *Athiorhodaceae* examined utilise several aromatic acids photosynthetically, some can also metabolise phloroglucinol in these conditions. Using *Rhodopseudomonas gelatinosa*, we detected dihydrophloroglucinol and 2-oxo-4-hydroxyadipate in photosynthetic cultures growing on phloroglucinol as sole carbon source<sup>25</sup>. Surprisingly, a soluble extract from these cells reduced phloroglucinol to dihydrophloroglucinol in the presence of NADPH as specific hydrogen donor in the dark. Our knowledge of the subsequent events leading to ring-fission is at present fragmentary, but is tentatively formulated as in Fig. 5.

### Anaerobic metabolism of benzoate through 'nitrate respiration'

Oshima<sup>26</sup> described a bacterial culture containing two different organisms from soil, which in combination but not separately, grew anaerobically on a variety of aromatic substrates in the obligatory presence of nitrate. Using  $\text{N}^{18}\text{O}_3^-$  and *p*-hydroxybenzoate or protocatechuate as substrate, these organisms incorporated more of the heavy oxygen isotope than with succinate as the C source. He concluded that in the anaerobic cleavage of the aromatic ring, the oxygen atoms of  $\text{NO}_3^-$  were used in a manner similar to that of molecular oxygen. This definitive inference seems unwarranted, bearing in mind the nature of his experiments. Taylor and his collaborators<sup>27,28</sup> then isolated from soil a pure culture of a *Pseudomonas* strain (PN 1) with a similar nitrate-dependent anaerobic metabolism of aromatic acids. Their results showed quite clearly that the anaerobic breakdown of the aromatic ring is different and quite distinct from the aerobic pathways. Although unsuccessful in revealing the pathway, they suggested that aromaticity was destroyed by the addition of three molecules of water to the benzene nucleus in preparation for ring

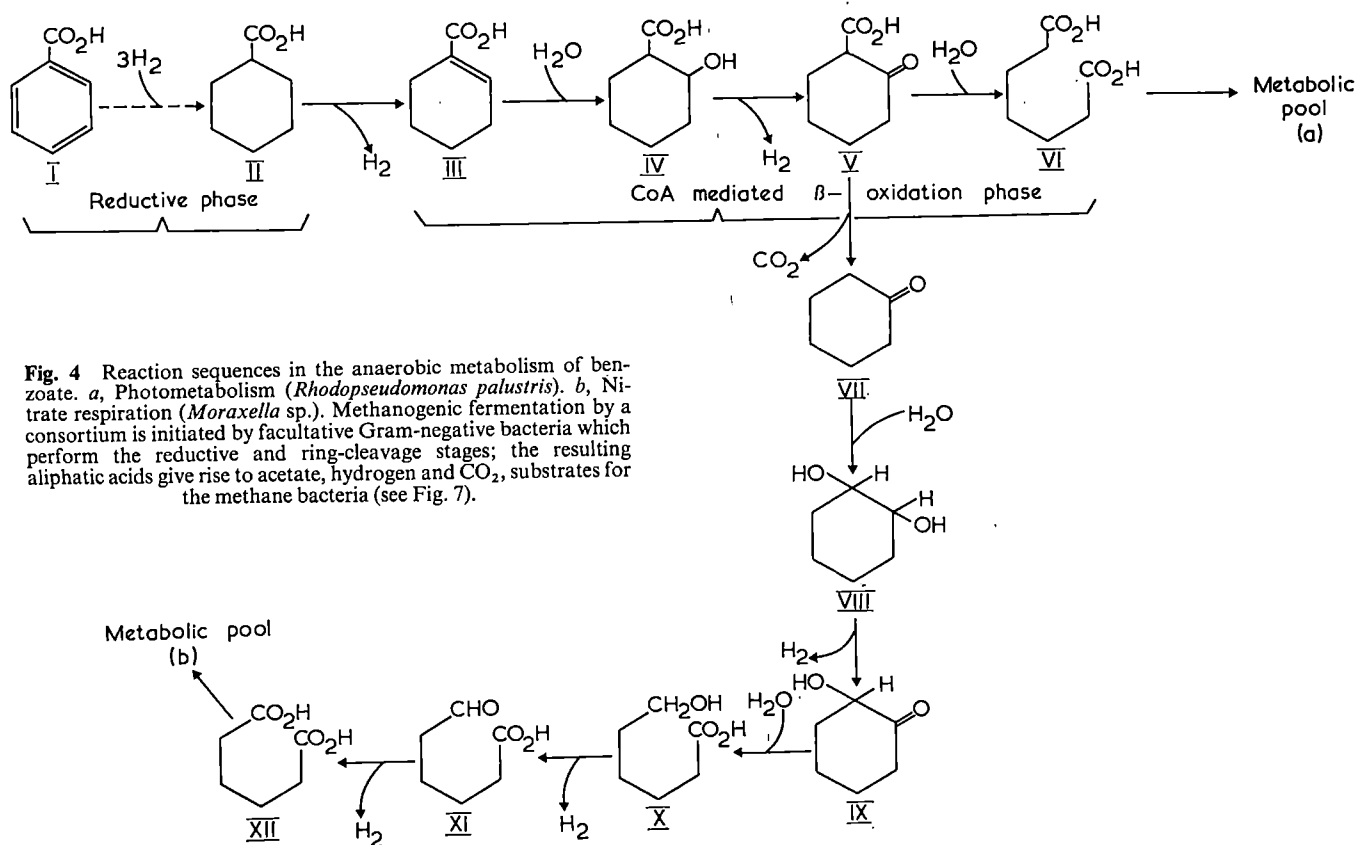


Fig. 4 Reaction sequences in the anaerobic metabolism of benzoate. a, Photometabolism (*Rhodopseudomonas palustris*). b, Nitrate respiration (*Moraxella* sp.). Methanogenic fermentation by a consortium is initiated by facultative Gram-negative bacteria which perform the reductive and ring-cleavage stages; the resulting aliphatic acids give rise to acetate, hydrogen and  $\text{CO}_2$ , substrates for the methane bacteria (see Fig. 7).

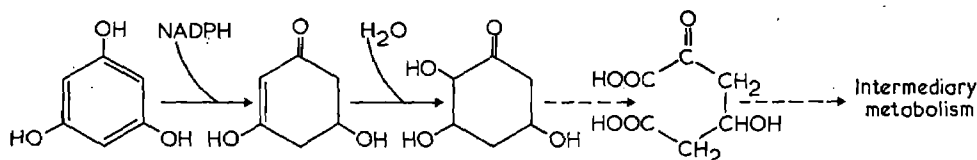


Fig. 5 Anaerobic metabolism of phenol by *Rhodopseudomonas gelatinosa*.

cleavage. (A similar mechanism had been suggested for the aerobic catabolism of aromatic substrates<sup>29</sup>.) We could not agree with their hypothesis on theoretical grounds, yet these workers had drawn attention to an important phenomenon. We also isolated a Gram-negative bacterium from soil which metabolised benzoate anaerobically in the presence of nitrate<sup>30,31</sup>; it turned out to be a *Moraxella* sp. (N.C.I.B.11086) instead of a *Pseudomonas*. Concomitant with the disappearance of the aromatic substrate in these *Moraxella* cultures, nitrate was reduced to nitrogen gas. When (ring-<sup>14</sup>C) benzoate was incubated anaerobically with these cells in nitrate-phosphate buffer, we identified labelled cyclohexanecarboxylate (II), cyclohex-1-enecarboxylate (III), 2-hydroxycyclohexanecarboxylate (IV) and adipate (XII). Using (carboxy-<sup>14</sup>C) benzoate in a similar experiment, the above intermediates were again radioactive with the exception of adipate. Subsequently, we were able to show that *Pseudomonas* PN1 (obtained through the courtesy of Dr Taylor) behaved similarly:

These results imply that the reductive pathway operates in the anaerobic catabolism of aromatic substrates through nitrate respiration<sup>31</sup>. The production of adipate, instead of pimelate in these cultures is explained by a divergence at the 2-oxocyclohexanecarboxylate stage, with its decarboxylation to cyclohexanone (VII) followed by alicyclic ring-cleavage by an unknown mechanism, as shown in Fig. 4.

Recently, Bakker<sup>32</sup> has demonstrated that a mixed bacterial culture adapted from a primary inoculum consisting of a mixture of soil, manure and sewage-sludge, degrades phenol in anaerobic conditions in a nitrate-mineral salts medium. The qualitative composition of this consortium remained fairly constant on serial transfer in liquid culture and consisted of Gram-negative bacteria and a spirillum. It also utilised benzoate, the monohydroxybenzoates, protocatechuate and the cresols in similar conditions. (ring-<sup>14</sup>C) Phenol was converted into <sup>14</sup>CO<sub>2</sub> and radioactive cell material; labelled *n*-caproate and acetate were also identified in the culture fluid. Bakker has, therefore, suggested that phenol is reduced to cyclohexanone followed by a hydrolytic scission of the alicyclic ring to *n*-caproate which can then undergo  $\beta$ -oxidation to give utilisable metabolites for cell growth, represented in Fig. 5. Methane was not produced in Bakker's cultures, presumably because of the presence of nitrate; Chmielowski and coworkers<sup>33</sup>, however, have described a methanogenic fermentation of phenol.

A probable interpretation of events in the bacterial catabolism of aromatic compounds through anaerobic nitrate respiration may be: the reductive phase is accomplished by a ferredoxin-type reductant followed by the  $\beta$ -oxidation sequence and ring-cleavage to aliphatic acids; since these have to serve as carbon and energy source, a part must be oxidised and the resulting reduced coenzymes re-oxidised via a membrane bound proton-translocating redox system which is coupled by the electron transport chain to nitrate through nitrate reductase.

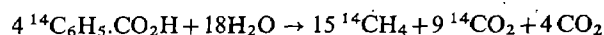
### Methanogenic fermentation by a consortium

The formation of methane and CO<sub>2</sub> from aromatic natural products was the first observation to be made involving the biological destruction of benzenoid structures in strictly anaerobic conditions<sup>18</sup>. It can occur in the absence of nitrate, sulphate

and light through the action of an adapted microbial community. It is surmised that this phenomenon occurs widely in nature, in the processing of sewage and other waste effluents and in transformations of some pesticides.

Clark and Fina<sup>34</sup>, Fina and Fiskin<sup>35</sup> and Roberts<sup>36</sup> working with benzoate as substrate and a consortium adapted from rumen-liquor and/or sewage-digester sludge, applied quantitative radiotracer techniques with the following results: (carboxy-<sup>14</sup>C) benzoate behaved like exogenous <sup>14</sup>CO<sub>2</sub> in that it was not primarily reduced to methane. (Ring-<sup>14</sup>C,1)benzoate appeared mainly as <sup>14</sup>CH<sub>4</sub>; (ring-<sup>14</sup>C,4) benzoate was converted largely to <sup>14</sup>CO<sub>2</sub>. Propionate, acetate and formate were detected in the steam volatile fatty acid fraction of the fermentation liquor. The propionate was labelled in the carboxyl-C when (ring-<sup>14</sup>C,4) benzoate was used (L. R. Fina, personal communication), but not when (ring-<sup>14</sup>C,1) or (carboxy-<sup>14</sup>C) benzoates were substrates.

That the composition of the fermentation gases agrees with the stoichiometry demanded by the equation below was confirmed by Nottingham and Hungate<sup>37</sup> using (ring-<sup>14</sup>C) benzoate:



Although several workers had shown that benzoate-methanogenic cultures utilised certain members of the reductive pathway without a lag period, definitive chemical evidence for their participation was lacking until an abstract of the work of Keith<sup>38</sup> appeared. Isotopic trapping experiments enabled him to identify cyclohexanecarboxylate, cyclohex-1-enecarboxylate, heptanoate, valerate, butyrate, propionate and acetate in such cultures. He proposed that benzoate is reduced step-wise to cyclohexanecarboxylate followed by a reductive cleavage of the C<sub>1</sub>-C<sub>2</sub> bond to give heptanoate;  $\beta$ -oxidation would then degrade this to valerate and propionate with the ultimate formation of acetate, one of the substrates for methane bacteria. Balba and Evans<sup>39</sup>, using similar techniques, also detected the formation of cyclohexanecarboxylate, cyclohex-1-enecarboxylate, propionate and acetate from benzoate in both rumen and sewage-sludge methanogenic cultures; adipate was also identified in the latter fermentation liquor. Although there were about five other unidentified labelled acidic components present, none of these corresponded to heptanoate, *n*-caproate, valerate or butyrate. Prins (personal communication) and his collaborators have identified *trans* 2-hydroxycyclohexanecarboxylate, 2-oxocyclohexanecarboxylate, pimelate, caproate, butyrate, acetate and molecular hydrogen as metabolites of benzoate in methanogenic cultures adapted from the black mud of a polluted river.

Whether the methanogenic fermentation of benzoate is accomplished entirely by species of methane bacteria or if it requires the cooperation of other members of the consortium, remained an open question until recently. In an elegant study, Ferry and Wolfe<sup>40</sup> observed that *o*-chlorobenzoate inhibited benzoate degradation without affecting the production of methane from acetate; the uncoupling of these two steps argues in favour of a microbial food chain. Quantitative studies on the rate of ap-

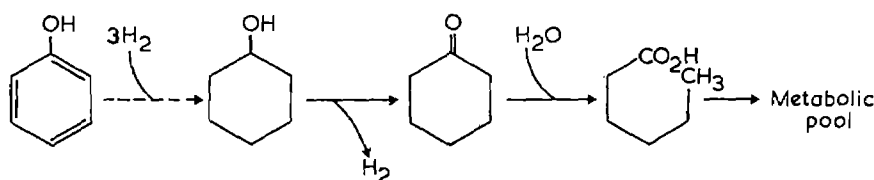
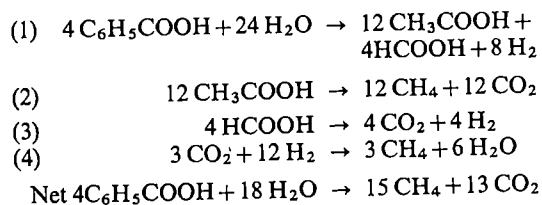


Fig. 6 Anaerobic metabolism of phenol through *n*-caproate by a consortium in the presence of nitrate. Initial reduction accomplished by an unidentified hydrogen donor (redox potential -0.42 V).

pearance and disappearance of intermediates and formation of products using  $^{14}\text{C}$  labelling where necessary, enabled these workers to propose the following steps in the overall conversion of benzoate to methane:



A careful study of their consortium which had been subcultured for a few years revealed three predominant methanogenic organisms. *Methanobacterium formicum* and *Methanospirillum hungatei* were obtained from the floc in pure culture, but the acetate-degrading methanogenic bacterium defied attempts at isolation. None of these utilised benzoate. A facultative Gram-negative organism which utilised benzoate aerobically was also present, but it failed to grow on this substrate anaerobically even in the presence of nitrate. Their cultures produced methane without a lag-period from cyclohexanecarboxylate, 2-hydroxy-cyclohexanecarboxylate, salicylate, pimelate and acetate; cyclohex-1-enecarboxylate did not behave this way. Ferry and Wolfe<sup>41</sup> point out that the energetics of benzoate degradation make it obligatory for the methane bacteria to utilise the acetate produced if the facultative benzoate-degrader is to obtain energy for growth in conditions which exist in the culture.

In the methanogenic cultures of Balba and Evans<sup>39</sup>, electron microscopy revealed methanogenic bacteria similar in morphology to those already described by Ferry and Wolfe<sup>40</sup>. The facultative Gram-negative organism present in our consortium grew aerobically on *p*-hydroxybenzoate but not on benzoate. It was, nevertheless, able to degrade benzoate anaerobically in the presence of nitrate (compare Williams and Evans<sup>31</sup>). Our cultures continued to produce a gas mixture of approximate composition,  $\text{CH}_4$  (54%),  $\text{CO}_2$  (46%) without a lag-period when benzoate was replaced by alicyclic intermediates of the reductive pathway, the hydroxybenzoates, phloroglucinol, cinnamate, phenylalanine and even shikimate or quinate.

It has now been established that in all these mixed cultures which ferment aromatic compounds to methane and  $\text{CO}_2$ , the benzene nucleus is first reduced and then cleaved to aliphatic acids by facultative Gram-negative organisms; these acids are then

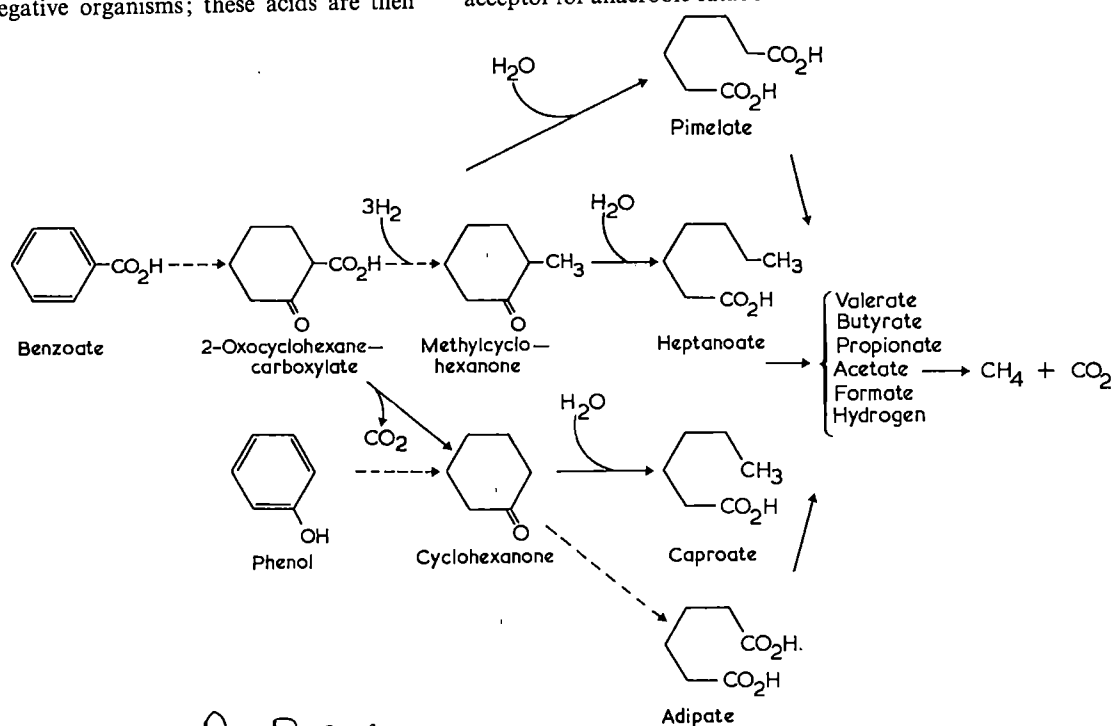
converted to suitable substrates for various methane bacteria to complete the process. The electrons generated are probably excreted as  $\text{H}_2$  gas which is used for the reduction of  $\text{CO}_2$  to  $\text{CH}_4$ . Pure cultures of all the necessary bacteria from these consortia are not yet available for the deliberate reconstitution of the community. It is unlikely that the consortia studied by different workers are absolutely identical in bacterial species composition, yet almost all investigators find the same alicyclic intermediates. The fatty acid anomaly, the occurrence of heptanoate in Keith's benzoate-methanogenic cultures could have other explanations for its origin rather than the one postulated, the reductive cleavage of cyclohexanecarboxylate, a reaction for which there is no biochemical precedent, Fig. 7 shows these intermediates.

Inocula from anaerobic environments where methane gas formation occurs, can be adapted to the methanogenic fermentation of benzoate in about 1–2 months; an initial addition of acetate as well to the culture shortens this period considerably. This does not mean that the benzene nucleus is actually degraded in all these anaerobic environments, for example, benzoate and the aromatic amino acids do not give rise to methane in the rumen; they are excreted as urinary hippurate and other conjugates. Shikimate and quinate are, however, reduced by rumen microflora to cyclohexanecarboxylate which appears in the urine of herbivores as hexahydrohippurate<sup>41</sup>.

The rate-limiting steps in the biological degradation of natural aromatic polymers occur at the stage when it is necessary to dismember the lignins and tannins into their small molecule aromatic components. (Artificial treatment of ligno-cellulosic material with alkali at an elevated temperature results in a much improved utilisation of their phenolic components by methanogenic fermentation<sup>42</sup>.) Extracellular microbial enzymes slowly achieve this aerobically, although there is a paucity of precise biochemical information about these reactions for obvious reasons<sup>43,44</sup>. The catechin tannins present an additional obstacle, because of their tanning properties.

It is difficult, at present, to assess the relative importance of the aerobic and anaerobic methods of decomposition of aromatic compounds in the biosphere. Regarding the latter, the *Athiorhodaceae* require light and anaerobiosis for it to be possible; such habitats exist in oceans and lakes<sup>45</sup>. Soils afford anaerobic conditions frequently generated by impeded drainage or flooding (natural or deliberate); soil aggregates (crumb structure) are anaerobic internally—where the appropriate bacteria reduce  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ . Whether  $\text{SO}_4^{2-}$  can also act as a terminal electron acceptor for anaerobic catabolism of aromatic substrates has not

Fig. 7 Probable pathways in the fermentation of benzoate and phenol by adapted bacterial consortia from a variety of methanogenic ecosystems. 1-Methylcyclohexanone is a hypothetical intermediate, but is one of a few possible precursors of heptanoate; others involve reduction of C.2 after a cleavage, or a biosynthetic origin.



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yet been convincingly demonstrated<sup>46</sup>; attempts in the authors' laboratory using *Desulphovibrio* were unsuccessful. Microbial communities in habitats where methane formation occurs abound in nature; they can also be made to produce the gas from a variety of aromatic substrates in the laboratory. Dutton and Evans<sup>47</sup> found that appreciable concentrations of fatty acids inhibited the photometabolism of benzoate—a temporary phenomenon, attributed at the time to competition for coenzyme A. In natural anaerobic environments, cellulose fermentation takes precedence over the degradation of aromatics.

Many factors are concerned in the degradation of organic compounds, irrespective of whether they are of natural or synthetic origin. They are all exposed to physico-chemical forces and biological agents capable of causing chemical change in the biosphere<sup>48</sup>. Dilution processes and time eventually ensure that conditions are created for these factors to operate. Concentrations of noxious chemicals may, however, accidentally reach levels in local areas which are extremely hazardous to life; these require exceptional decontamination measures and legislation. Although many useful pesticides are developed empirically, their mode of action usually depends on a selective interference with some fundamental cellular process. Their structures, apart from certain uncommon substituents (for example, halogen) also frequently bear some resemblance to key biochemicals concerned in metabolism. Some linkages may, therefore, be amenable to attack by constitutive group-specific microbial enzymes. It is a common observation that many of these foreign chemicals are degraded more rapidly by a mixed adapted community of soil microorganisms than by any of the freshly isolated individual members of the group—for example many chlorinated hydrocarbon pesticides under suitable biologically-active anaerobic conditions<sup>49,50,51</sup>; Dalapon<sup>52</sup> (2, 2-Dichloropropionate) and Asulam (M. Khan and W.C.E., unpublished) [Methyl-(4-aminobenzenesulphonyl)carbamate] under aerobic conditions.

A microbial community reacts to the presence of xenobiotic organic chemicals in subtle ways, depending on their structures and the environment; several biological devices are brought into play which ultimately cause their disappearance. Among the known stratagems used are: co-metabolism<sup>53</sup>, enzyme induction<sup>54</sup>, transfer of metabolic plasmids<sup>55,56</sup>, mutation leading to the evolution of enzymes with new specificities and activities<sup>57,58</sup> and microbial interactions<sup>59</sup> as yet imperfectly understood but operating in the methanogenic consortia which anaerobically degrade aromatic substrates.

This work was supported by the SRC. I thank all my collaborators for their contributions.

1. Jenkinson, D. S. *Biochem. J.* **109**, 2P (1968).
2. Zobell, C. in *Proc. Rudolf's Research Conf: Principles and Applications in Aquatic Microbiology* (eds Heukeljian, A. & Dondoro, N. C.) 337-339 (Wiley, New York, London and Sydney 1964).
3. McLaren, A. D. *Science* **141**, 1141-1147 (1963).
4. Evans, W. C. & Happold, F. C. *J. Soc. Chem. Industr.* **58**, 55 (1939).
5. Evans, W. C. *Biochem. J.* **41**, 373-382 (1947).
6. Kilby, B. A. *Biochem. J.* **43**, v (1948).
7. Hayaishi, O. & Hashimoto, K. *J. Biochem. Tokyo*, **37**, 371-374 (1950).
8. Evans, W. C., Smith, B. S. W., Linstead, R. P. & Elvidge, J. A. *Nature* **168**, 772-775 (1951).
9. Dagley, S. & Stopher, D. A. *Biochem. J.* **73**, 16P-17P (1959).
10. Ribbons, D. W. & Evans, W. C. *Biochem. J.* **76**, 310-316 (1960).
11. Trippett, S., Dagley, S. & Stopher, D. A. *Biochem. J.* **76**, 9P (1960).
12. Dagley, S., Evans, W. C. & Ribbons, D. W. *Nature* **188**, 560-566 (1960).
13. Hayaishi, O. *Plenary Sessions Sixth Internat. Congr. Biochemistry, New York City I.U.B. 33*, 31 (1964).
14. Dagley, S. *Essays Biochem.* **11**, 81-138 (1975).
15. Williams, P. A. *Biochem. Soc. Trans.* **4**, 452-473 (1976).
16. Kieslich, K. *Microbial Transformations of Non-Steroid Cyclic Compounds*, 1-1262 (Wiley, New York & London, 1976).
17. Dutton, P. L. & Evans, W. C. *Biochem. J.* **109**, 5P (1968).
18. Tarvin, D. & Buswell, A. M. *J. Am. chem. Soc.* **56**, 1751-1755 (1934).
19. Proctor, M. H. & Scher, S. *Biochem. J.* **76**, 33P (1960).
20. van Niel, C. B. *Adv. Enzymol.* **1**, 263-328 (1941).
21. Leadbetter, E. R. & Hawk, A. *J. appl. Bacteriol.* **27**, 448 (1965).
22. Dutton, P. L. & Evans, W. C. *Biochem. J.* **104**, 30P (1967).
23. Dutton, P. L. & Evans, W. C. *Biochem. J.* **113**, 525-536 (1969).
24. Guyer, M. & Hegeman, G. D. *J. Bacteriol.* **99**, 906-907 (1969).
25. Whittle, P. J., Lunt, D. O. & Evans, W. C. *Biochem. Soc. Trans.* **4**, 490-491 (1976).
26. Oshima, T. *Allg. Mikrobiol.* **5**, 386-394 (1965).
27. Taylor, B. F., Campbell, W. L. & Chinoy, I. J. *Bacteriol.* **102**, 430-7 (1970).
28. Taylor, B. F. & Heeb, M. J. *Arch. Mikrobiol.* **83**, 165-171 (1972).
29. Stanier, R. Y. *J. Bacteriol.* **55**, 477-494 (1948).
30. Williams, R. J. & Evans, W. C. *Biochem. Soc. Trans.* **1**, 186-187 (1973).
31. Williams, R. J. & Evans, W. C. *Biochem. J.* **148**, 1-10 (1975).
32. Bakker, G. *FEMS Lett.* **1**, 103-108 (1977).
33. Chmielowski, J., Grossman, A. & Węgrzynowska, I. *Zeszyty Naukowe Politechniki Śląskiej. Inżynieria Sanitarna*, **8**, 97-122 (1964).
34. Clark, F. M. & Fina, L. R. *Archs Biochem. Biophys.* **36**, 26-32 (1952).
35. Fina, L. R. & Fiskin, A. M. *Archs Biochem. Biophys.* **91**, 163-165 (1960).
36. Roberts, F. F. thesis, Kansas State Univ. (1962).
37. Nottingham, P. M. & Hungate, R. E. *J. Bacteriol.* **98**, 1170-1172 (1969).
38. Keith, C. L. *Dissertations Abstr. Intern.* **B. 33**, 3214-3215 (1972).
39. Balba, M. T. & Evans, W. C. *Biochem. Soc. Trans.* **5**, 302-304 (1977).
40. Ferry, J. G. & Wolfe, R. S. *Arch. Mikrobiol.* **107**, 33-40 (1976).
41. Balba, M. T. & Evans, W. C. *Biochem. Soc. Trans.* **5**, 300-302 (1977).
42. McCarty, P. L. *et al. Microbiol Energy Conversion* (eds Schlegel, H. G. & Barnes, J.) 179-199 (UNITAR, Göttingen, 1977).
43. Sundman, V. *Acta Polytechnica Scand.* **40**, 1-116 (1965).
44. Trojanowski, J. *Acta Microbiol. Polonica* **2**, 13-22 (1970).
45. Stewart, W. D. P. *Algae, Man and the Environment* (ed. Jackson, D. F.) 53-72 (Syracuse University Press, Syracuse, 1968).
46. Einmihjellen, K. *Proc. 3rd Inter. Congr. Photobiol.*, S. Scher. Quoted in footnote, 583-585 (1960).
47. Dutton, P. L. & Evans, W. C. *Archs Biochem. Biophys.* **136**, 228-232 (1970).
48. *The Persistence of Insecticides and Herbicides Br. Council Crop Protection Mon. No 17*. (ed. Beynon, K. I.) (Boots, Nottingham, 1976).
49. Raghu, K. & Macrae, I. C. *Science* **19**, 263-264 (1966).
50. Hill, D. W. & McCarty, P. L. *J. Water Poll. Control* **39**, 1259-1277 (1967).
51. Ahmed, M. K., Casida, J. E. & Nichols, R. E. *Agric. Food Chem.* **6**, 740-745 (1958).
52. Senior, E., Bull, A. T. & Slater, J. H. *Nature* **263**, 476-479 (1976).
53. Horvath, R. S. *Bacteriol. Rev.* **36**, 146-155 (1972).
54. Cohn, M. *et al. Nature* **172**, 1096 (1953).
55. Williams, P. A. & Worsey, M. J. *Biochem. Soc. Trans.* **4**, 466-468 (1976).
56. Pemberton, J. M. & Fisher, P. R. *Nature* **268**, 732 (1977).
57. Clarke, P. H. in *Evolution in the Microbial World* (ed. Carlile, M. J. and Skehel, J. J.) (Cambridge University Press, London, 1974).
58. Hartley, B. S. in *Evolution in the Microbial World* (ed. Carlile, M. J. and Skehel, J. J.) (Cambridge University Press, London, 1974).
59. Bryant, M. P., Wolin, E. A., Wolin, M. J. & Wolfe, R. S. *Arch. Mikrobiol.* **59**, 20-31 (1967).

## articles

# Multichannel seismic reflection profiles of the continental crust beneath the Newfoundland Ridge

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*Multichannel seismic reflection profiles extending south from the Grand Banks indicate that large 'basement' features underlying the Newfoundland Ridge are composed of sedimentary strata. The Newfoundland Ridge, therefore, is interpreted as an area of subsided continental crust rather than a ridge of oceanic crust. The J magnetic anomaly intersects this complex, and the physiographic ridge associated with this anomaly may also be continental in origin.*

HYPOTHETICAL reconstructions of the North Atlantic before Mesozoic-Cainozoic seafloor spreading define the south-western edge of the Grand Banks structural block as a transform margin that developed when the African plate was displaced from that region<sup>1,2</sup>. The Newfoundland Ridge (Fig. 1) has been interpreted as the trace of a fracture zone in oceanic crust marking the continuation of this transform margin<sup>3,4</sup>. The Newfoundland Ridge is a physiographic feature that extends about 900 km south-east from the southern tip of the Grand Banks, with a subsidiary projection, the 'Spur' ridge or 'J-anomaly' ridge, extending to the

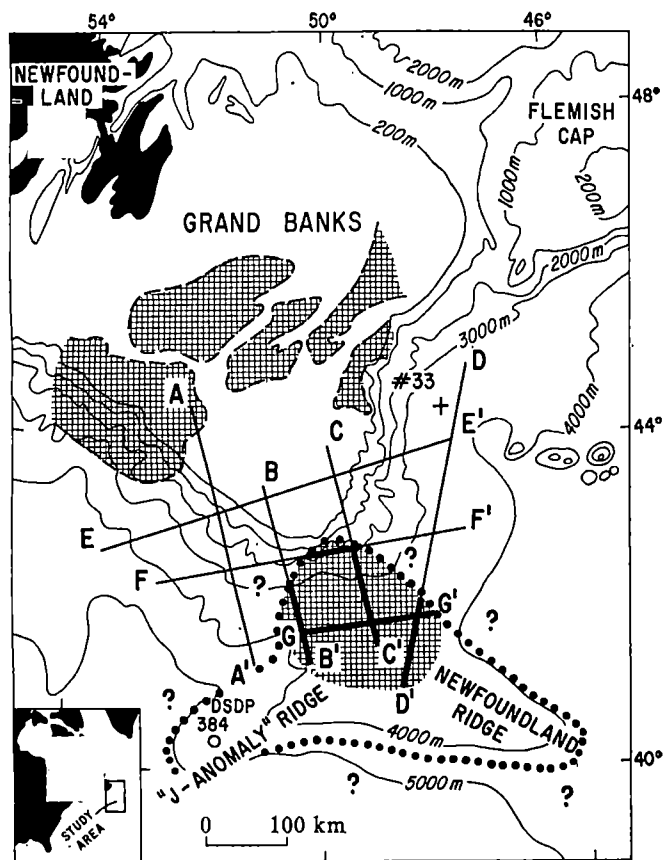


Fig. 1 Bathymetric map of the Grand Banks region (contours in metres). Lettered lines show the locations of the profiles drawn in Fig. 2. The heavy parts of these lines and surrounding crosshatch denote areas where seismic reflectors are apparent beneath event U. The dotted line indicates the minimum extent of sedimentary strata beneath the Newfoundland Ridge inferred on the basis of physiography. Question marks denote the possibility of founded sialic crust adjacent. Cross-hatched areas enclosed by dashed lines indicate the extent of sub-unconformity basins of the Grand Banks. The circle and cross indicate DSDP Site 384 (ref. 11) and sonobuoy station No. 33 (ref. 10).

south-west. Watson and Johnson<sup>5</sup> interpreted seismic profiles from the Newfoundland Ridge as showing buried basement blocks, which had been uplifted and distorted by faulting. From the magnetic anomalies associated with these blocks they inferred a volcanic origin. Ballard *et al.*<sup>6</sup> have described topographic and structural features characteristically associated with the high amplitude (500–1,000 nT) J-magnetic anomaly. Hall<sup>7</sup> and Keen *et al.*<sup>8</sup> have suggested that the J-magnetic anomaly continues north-eastwards with slight offset, across the Newfoundland Ridge. These investigations generally imply an oceanic origin for the Newfoundland Ridge. This paper presents an interpretation of multichannel (24) common depth point reflection seismic data that supports an alternative, continental origin for this feature. These seismic data (Fig. 1) are part of a nonproprietary survey by Seiscan Delta Ltd that the Canadian Government has purchased for the purpose of regional control. Figure 2 shows reduced tracings of reflectors on the processed record sections. The vertical scale magnification of the bottom profile in these diagrams is about 6 to 1. Magnetic data (also from Seiscan Delta) are plotted as anomaly profiles with several of the seismic sections. Figure 3 is a reproduction of a portion of a seismic record section on the Newfoundland Ridge.

### Interpretation of data

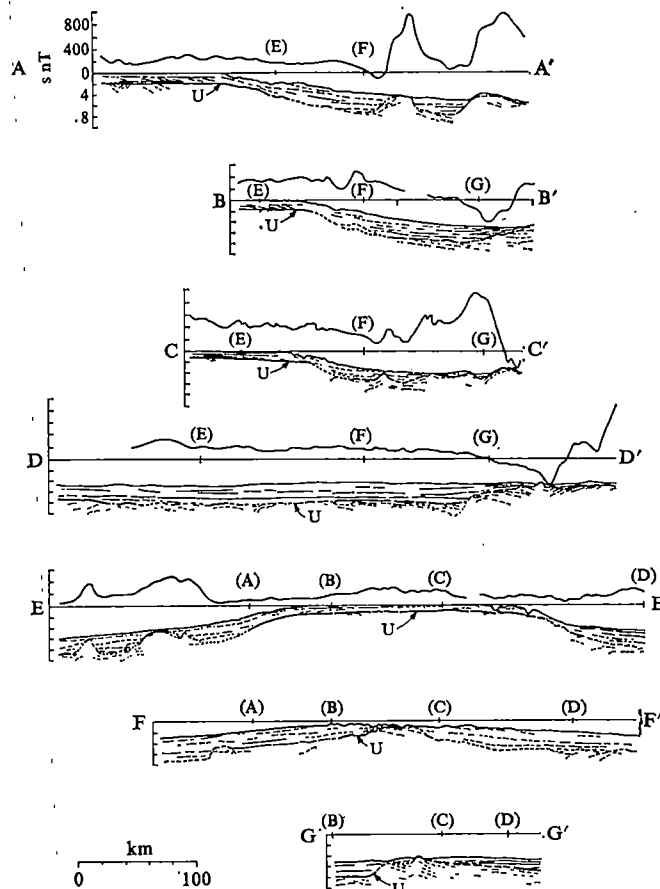
The subsurface geology of the Grand Banks has been outlined through geophysical surveys and exploratory drilling for petroleum<sup>1,9</sup>. A principal element of Grand Banks geology is a late

early Cretaceous unconformity, which separates a blanket of Cretaceous and Tertiary sediments from older rocks in sub-unconformity basins and intervening areas of basement (Fig. 1). This unconformity is an excellent seismic boundary. On the profiles in Fig. 2 the seismic reflections from the unconformity—(event U) have been traced from the shelf to deep water. On individual seismic records it is usually difficult to follow this event through the zone of the shelf break, where the change in bottom slope and probable changes in sediment lithology combine to degrade record quality. Fortunately, however, the correlation of the U event from the shelf to deep water can be checked by crossreferencing the intersecting lines of survey coverage (Fig. 1).

Section G and the southern ends of sections B to D (Fig. 2), located on the Newfoundland Ridge, show seismic reflectors to depths approaching 4 s (two-way time) below the U event, with no indication of any underlying basement. These sub-U reflectors are interpreted as denoting gently dipping sedimentary strata. Velocity analyses performed in the course of data processing indicate velocities in these strata as low as 3–4 km s<sup>-1</sup>, which would yield a minimum thickness for these strata of 6–8 km. The apparent attitudes of the seismic reflectors at the crossing of lines C and D with line G indicate a north-westerly strike, approximately parallel to the physiographic trend of the Newfoundland Ridge. These strata form cuestas and hogbacks at the U event interface; however, it is not clear from the seismic data whether the scarps associated with these features are entirely erosional or whether displacement by faulting has occurred. Dipping reflectors below the U event in the central part of line F, between crossings with lines B and C, are also interpreted as originating in sedimentary strata.

Sub-U penetration of a different character occurs on the eastern end of line E, and along the northern three-quarters of line D (Fig.

Fig. 2 Seismic and magnetic profiles from locations indicated in Fig. 1. Vertical scale for the seismic profiles is in seconds of two-way travel time. The U event has been emphasised. Vertical bars on profile B indicate coverage of Fig. 3.



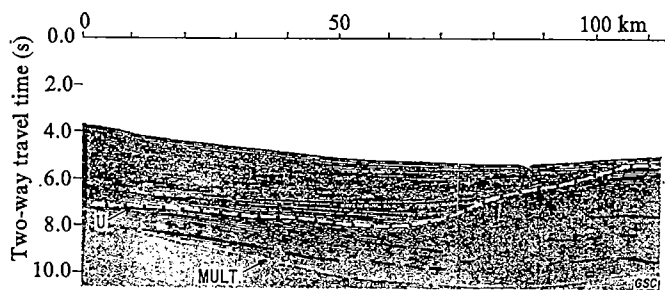


Fig. 3 Photograph of a portion of a seismic record section on the Newfoundland Ridge.

2). These reflectors show variable attitudes, amplitudes and continuity. Beneath the south-western slope of the Grand Banks, large anomalies traced by the U event on lines A and E have been interpreted by Jackson *et al.*<sup>10</sup> as relief on oceanic basement. The seismic record sections show reflectors on the flanks of these features, but no apparent penetration beneath their central, highest parts. The character and disposition of these flanking reflectors give the impression of talus deposits.

The reflection seismic data described above distinguish between acoustic basement highs beneath the south-western slope of the Grand Banks and blocks of sedimentary strata beneath the Newfoundland Ridge. Available magnetic coverage<sup>7</sup> shows a complex assemblage of large magnetic anomalies associated with both areas, and with the J-anomaly ridge as well, but does not seem adequate to refine the limits of these two types of sub-U structure indicated by seismic data. Possibly there is a north-westerly trend to the magnetic anomalies in the vicinity of the Newfoundland Ridge.

Occasional penetration of seismic energy is seen beneath the U event on the Grand Banks (sections A, B, C and E, Fig. 2). The north end of section A shows more extensive penetration associated with the South Whale sub-basin (Fig. 1). Excellent seismic records across the sub-unconformity basins on the Grand Banks have been presented by Amoco and Imperial<sup>9</sup>.

## Discussion

Assuming that the U seismic event has been traced correctly from the Grand Banks to the limit of coverage in deep water, and that the chronological significance of this event remains approximately constant, then the age of the sedimentary strata underlying the U event on the Newfoundland Ridge must be early Cretaceous or older. The U event unconformity is evidence that these strata were exposed to subaerial weathering before the subsidence time. The results from DSDP site 384, on the J-anomaly ridge (Fig. 1), indicate that subsidence of at least 4 km has affected this area since the late early Cretaceous<sup>11</sup>. The apparent thickness of the strata beneath the U event on the Newfoundland Ridge (up to 6–8 km) is comparable to the thickness of section that occurs in the sub-unconformity basins on the Grand Banks, and the areal extent of these strata as defined by the seismic lines in Fig. 2 is at least equivalent to that of the smaller basins on the Grand Banks (Fig. 1). It is, therefore, postulated that the sedimentary strata underlying the U event on the Newfoundland Ridge may represent a basinal accumulation analogous to the sub-unconformity basins on the Grand Banks, which has subsided relative to the Grand Banks by displacement across a hinge line or zone of faulting beneath the present continental slope. If these strata extend to the physiographic limit of the Newfoundland Ridge (Fig. 1), this feature would represent a subsided, remnant basin roughly equivalent in area to the total of the several remnant basins on the Grand Banks.

The sub-unconformity basins on the Grand Banks are fault-bounded grabens and half-grabens containing Jurassic and older formations, with structural complications related to salt diapirism<sup>9</sup>. The sparse data available indicate that the basement rocks underlying these formations are composed of a wide variety of lithologies and probably range in age from Palaeozoic to

Precambrian<sup>1,9</sup>. These data are a meagre basis for speculation as to the nature of the basement rocks that underlie the sub-U sedimentary strata on the Newfoundland Ridge, except that they are probably sialic. But, an important implication of the inferred basinal setting of these strata is that the basement rocks adjacent to the basin subsided more deeply than the sediments, and that foundered sialic rocks thus extend some distance laterally from the Newfoundland Ridge.

The multichannel seismic coverage (Fig. 1) is not sufficient to determine the extent of sialic crust laterally from the Newfoundland Ridge, but variations in the character of these seismic data in the area peripheral to the ridge may be relevant. Off the eastern margin of the Grand Banks the northern three-quarters of line D (Fig. 2) shows irregular and discontinuous reflectors beneath event U. The depth to the U event opposite sonobuoy Station No. 33 (Fig. 1) of Jackson *et al.*<sup>10</sup> corresponds closely to the depth to their inferred oceanic basement refractor of  $5.82 \text{ km s}^{-1}$ . On the south-western side of the Grand Banks Jackson *et al.*<sup>10</sup> recorded a mantle refraction at a depth of approximately 17 km, and described the irregular surface traced by the U event in that area (lines A and E, Fig. 2) as oceanic basement. The refraction seismic definition of oceanic basement in these two areas obviously is not sensitive to the contrasts in surface profile and seismic penetration shown by the multichannel reflection seismic records, and it is doubtful that both of these refraction measurements define true oceanic basement. Possibly the differences in the reflection seismic character of these two areas relate to variation in the composition of parent sialic crust, now subsided.

The extent of subsided continental crust peripheral to the Newfoundland Ridge can be examined in a larger context. Pre-drift fits of the North Atlantic tend to place the western margin of the Iberian Peninsula opposite the south-western margin of the Grand Banks<sup>1,2</sup>. In some reconstructions, Galicia Bank, which lies offshore west of the northern part of the Iberian Peninsula, obstructs the tight juxtaposition of these two margins and leaves an untidy gap to the south. An area of continental crust about the Newfoundland Ridge would help to fill this gap.

The large magnetic anomalies in the vicinity of the Newfoundland Ridge may relate to the presence of subsided continental crust as hypothesised above. Possibly these large anomalies express the susceptibility contrast between sedimentary rocks and volcanic rocks, assuming that the sediments and sialic basement rocks were invaded by mafic volcanics, probably at the time of subsidence. This interpretation may also apply to the physiographic and magnetic characteristics of the J-anomaly ridge, which coincides with a larger magnetic anomaly than expected in relation to its physiographic relief and reasonable magnetisation values for oceanic basement<sup>11</sup>. The distinctive topographic aspects of the J-anomaly ridge<sup>6</sup>, particularly the anomalously flat surfaces apparently disrupted by faults, would be normal features for a ridge composed primarily of sedimentary strata. This postulated origin for the J-anomaly ridge implies that it does not necessarily have an isochronal relationship to seafloor spreading. Hall<sup>7</sup> and Keen *et al.*<sup>8</sup> have discussed the problematic nature of the age of the J anomaly, which has been assigned ages ranging from 115 to 137 Myr BP.

## Conclusions

Multichannel reflection seismic data from the Newfoundland Ridge allow penetration below the surface commonly regarded as oceanic basement, with important consequences for the geological interpretation of this region. These data indicate that the Newfoundland Ridge is composed of blocks of sedimentary strata, pre-late early Cretaceous in age, which may represent a sedimentary basin, now subsided, analogous to the sub-unconformity basins on the Grand Banks.

A synthesis of available data from the region of the southern Grand Banks suggests that sialic crust adjacent to the Newfoundland Ridge has subsided to oceanic depths, and that variations in its composition are shown by variation in reflection seismic character. These differences are beyond the resolution capability of the refraction seismic method. If it is accepted that the

subsidence of sialic crust was accompanied by igneous invasion, a new pattern of magnetic anomalies probably was generated, with their orientation determined by the mechanical processes of subsidence and the geological fabric of the parent crustal material. These anomalies would not be expected to have chronological significance in terms of processes of seafloor spreading.

Additional survey control is required to map the limits of postulated subsided continental crust in the vicinity of the Newfoundland Ridge. Probably it will be necessary to apply common depth point reflection seismic techniques to effectively pursue this problem. Increased application of the common depth point reflection seismic method in the deep sea, particularly in the marginal zones of the ocean basins, will reveal further variations in the character of oceanic basement. The data described here indicate another probable sliver, chip or mini-plate that geomet-

ricians must accommodate in closing the North Atlantic to predrift configurations.

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1. Jansa, L. F. & Wade, J. A. *Geol. Surv. Can. Paper* 74-30, 2, 51-105 (1975).
2. Williams, C. A. *Earth planet. Sci. Lett.* 4, 440-456 (1975).
3. Auzende, J. M., Olivet, L. L. & Bonnin, S. *CR Acad. Sci.* 271 (D), 1063-1066 (1970).
4. Le Pichon, X. & Fox, P. J. *J. geophys. Res.* 76, 6294-6308 (1971).
5. Watson, J. A. & Johnson, G. L. *Can. J. Earth Sci.* 7, 306-316 (1970).
6. Ballard, J. A., Vogt, P. R. & Egloff, G. *Trans. Am. geophys. Union* 57, 264 (1976).
7. Hall, B. R. thesis, Dalhousie Univ. (1977).
8. Keen, C. E., Hall, B. R. & Sullivan, K. D. *Earth planet. Sci. Lett.* (in the press).
9. Amoco Canada Petrol. Co. Ltd. & Imperial Oil Ltd. *Bull. Can. petrol. Geol.* 21, 479-503 (1973).
10. Jackson, H. R., Keen, C. E. & Keen, M. J. *Geol. Surv. Can. Paper* 74-30, 1-13 (1975).
11. Tucholke, B., Vogt, P. R. & Scientific Party *Geotimes* 20, 18-31 (1975).

# Absolute radiocarbon dating using a low altitude European tree-ring calibration

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*Attempts to produce a calibration curve for the radiocarbon timescale by analysis of known age materials have highlighted the inaccuracies of conventional radiocarbon dating methods. The resulting ambiguities have caused a loss of confidence in radiocarbon dating particularly among European pre-historians. We describe here an absolute radiometric dating technique involving the investigation of all probable sources of error. Accurate measurements were made with an overall precision of less than 25 yr standard deviation, on a floating North of Ireland tree-ring chronology.*

CORRECTION for natural atmospheric radiocarbon variation is only one of the several corrections that have been necessary to relate the radiocarbon assay of organic materials to a calendar time scale. The assumption of the constancy of natural radiocarbon was first questioned by DeVries<sup>1</sup>, and several teams set out to test the constancy by measuring the radiocarbon content of known age samples. Willis, Tauber and Munnich<sup>2</sup> and Suess<sup>3</sup> showed that there was a significant trend away from the theoretical relationship. The bristlecone pine chronology was used for further investigation of the problem<sup>4,5</sup>. Suess showed major short-term variations or 'wiggles' in the calibration and suggested that in certain periods radiocarbon measurements could not provide unambiguous dates.

Further bristlecone pine samples were then distributed to three laboratories; La Jolla, Arizona and Pennsylvania and the results presented in 1969<sup>6</sup>. Suess<sup>7</sup> presented his calibration as a curve drawn by eye through the measurement points, incorporating a number of pronounced wiggles. Part of this graph is reproduced in Fig. 1. Following publication several authors<sup>8-11</sup> questioned the validity of Suess's line.

During the next few years few new data were produced from the radiocarbon laboratories and discussions continued among radiocarbon users on the validity of the wiggles and on what methods should be used to apply corrections to radiocarbon age measurements<sup>12,13</sup>. At various times the bristlecone pine tree-ring chronology was questioned, but this was resolved by the replication of the greater part by LaMarche and Harlan<sup>14</sup>. The possibility was also raised that *in situ* production of <sup>14</sup>C in the high altitude bristlecone pines was leading to spurious results. This has been discounted both by experiment<sup>15</sup> and by theoretical calculations based on the nitrogen content of the wood<sup>16</sup>. Two more recent calibrations are given by Damon *et al.*<sup>17</sup> who present a

calibration based on 549 samples using a curvilinear regression, and by Clark<sup>13</sup>. The latter summarises previous calibrations and examines the evidence from duplicate measurements which indicate a greater spread in the results than can be accounted for by the stated precisions. Disagreements between radiocarbon dates for bristlecone pine wood and for same-age, historically dated, Egyptian material are apparent. These disagreements led McKerrell<sup>18</sup> to suggest that systematic differences may exist and that the bristlecone calibration may not be appropriate in European contexts.

Although it is 19 years since the DeVries report, there is still no satisfactory experimentally-derived calibration that can be used unambiguously to convert radiocarbon ages to calendar dates. The publication of numerous calibration curves based on identical material, has proved that laboratories can only agree on the general trend of the calibration. Because of these disagreements it has been left to statisticians to provide smoothed calibrations. One such attempt has been the fit by Damon<sup>17</sup> of a third order orthogonal polynomial to experimentally derived data. At a recent conference no internationally acceptable radiocarbon calibration could be arrived at. It was apparent that an attempt should be made to resolve these discrepancies on the assumption that the experimental method was at fault.

By 1970 studies on oaks had established that long tree-ring chronologies could be constructed in the North of Ireland. To exploit this source of low altitude calibration material, a radiocarbon dating system capable of taking advantage of large sample size was designed; the method chosen was the liquid scintillation counting of benzene synthesised from sample cellulose. In 1972 a research programme was started to investigate all factors likely to contribute to the inaccuracy of radiocarbon measurement. Details of the procedure for synthesising large volume benzene samples and methods of counting will be given elsewhere (G.W.P., in preparation). So that the results given here may be critically examined, the corrections derived from these investigations are given in Table 1.

## Methods

The measurements were carried out on a 1,200-yr section of a 2,990-yr floating tree-ring chronology derived from sub-fossil oaks which grew below 130 m altitude between latitude 54° and 55°N and longitude 6° and 8°W (ref. 19). The chronology is based on some 300 trees and is replicated at all points. Because of bulk-field sampling and relatively large annual rings it was possible to produce 20-yr units of 180-200 g for radiocarbon measurement. This is in comparison with normal bristlecone pine samples of 20 g.

Table 1 Experimentally derived corrections for significant error sources

Error source investigated	Correction derived experimentally	Normalised value	Estimated error on correction
Barometric pressure variation	Background increases by 0.0127 c.p.m. per mbar decrease in* pressure	1010 mbar	$\pm 0.013$ c.p.m.
Background variations with weight loss	Background decreases by 0.0003 c.p.m. per mg decrease in weight	13.1325 g C <sub>6</sub> H <sub>6</sub> 14.2666 g C <sub>6</sub> H <sub>6</sub> + scintillant	$\pm 0.003$ c.p.m.
Background variations with purity	Changes by 0.041 c.p.m. per cent change in channels ratio	External source channels ratio of 1.95	$\pm 0.041$ c.p.m.
Efficiency variation with weight loss	Efficiency increases by 0.00104% per mg decreases in weight	13.1325 g C <sub>6</sub> H <sub>6</sub> 14.2666 g C <sub>6</sub> H <sub>6</sub> + scintillant	$\pm 0.010\%$ of nett c.p.m.
Differential loss of sample from mixture and correction to normalised weight including all weighing errors†	98.3% of loss from vial is sample benzene	13.1325 g C <sub>6</sub> H <sub>6</sub>	$\pm 0.076\%$ of nett c.p.m.
Efficiency variation with purity	‡%Deviation from normal purity 0.0–1.0 (1.00005) 1.0–2.0 (1.00015) 2.0–3.0 (1.00025)	External source channel ratio of 1.95	$\pm 0.005\%$ of nett c.p.m.
Mass spectrometry correction	Isotopic enrichment measured (actual reproducibility over 12 months)	–25% rel. P.D.B. (samples) –19% rel. P.D.B. (oxalic reference)	$\pm 0.05\%$ of nett c.p.m. $\pm 0.05\%$ of nett c.p.m.
Individual vial difference for efficiency	Using high <sup>14</sup> C labelled C <sub>6</sub> H <sub>6</sub>	Mean value	$\pm 0.029\%$ of nett c.p.m.
Individual vial difference for background	95% limits of parent distribution	Mean value	$\pm 0.058$ c.p.m.

The method used for calculation was that given by Callow *et al.*<sup>27</sup>.

\*High correlation of background with barometric pressure was found. Failure to correct could lead to a bias of up to 100 yr and make use of long-term mean background values impossible.

†Large inaccuracies, up to 40 mg, can be caused by the effect of humidity and temperature on plastic caps.

‡Correction factor given in parentheses.

Measurements were made on contiguous 20-yr increments taken from different trees in the floating chronology. Each increment was reduced to shavings and treated with a chlorite bleach<sup>20</sup> which left a sample free from tannins and lignins and close to pure cellulose. This treatment has been shown<sup>16</sup> to remove the greater part of any effect of heartwood deposition in oak since cellulose is not laid down at the heartwood/sapwood transition. Following cellulose preparation samples were charred at 450 °C to leave a carbon residue and converted to CO<sub>2</sub> by combustion or bomb calorimetry. The gas was sub-sampled for mass spectrometric measurement of the stable carbon isotope ratio.

The synthesis of benzene from sample CO<sub>2</sub> followed the conversion path CO<sub>2</sub> → LiC → C<sub>2</sub>H<sub>2</sub> → C<sub>6</sub>H<sub>6</sub> using the method of Barker<sup>21</sup>. This method was modified to allow the synthesis of up to 20 ml of sample benzene. Contamination of the water supply, and of the lithium metal used in the synthesis with <sup>222</sup>Rn was severe and great care was needed to ensure its removal. Mass spectrometric analysis of the synthesised benzene showed that it was at least as pure as scintillation grade benzene and was of consistent quality.

The samples were measured on a Philips 4510 automatic liquid scintillation analyser with the three channels for individual isotope monitoring set for the measurement of <sup>3</sup>H, <sup>14</sup>C and <sup>222</sup>Rn. Two extra channels were used for the differential energy measurement of a 7 μCi <sup>133</sup>Ba external source, the ratio being used to check variation in pulse height (a limit of  $\pm 4.5\%$  of mean purity pulse height was applied). Balance point counting of <sup>14</sup>C (a differential technique in which the pulse height spectrum within the selected channel is so balanced that small variations in pulse height contribute almost as many pulses to the channel as are lost) reduced the effect of pulse height variation giving long-term changes in efficiency of less than 0.03%. Continuous background

measurement of a flame-sealed vial showed no significant deviation from a normal distribution when corrected for barometric pressure variation (a major source of error, see Table 1). Gain stability checks were maintained using flame-sealed vials containing high specific activity <sup>3</sup>H and <sup>14</sup>C-labelled toluene. Because of the relative increase in pulse height of toluene to benzene the <sup>14</sup>C high standard was not measured in a balance point position and was therefore very sensitive to gain changes. The <sup>14</sup>C channel was set so that balance point was achieved at a specific purity equivalent to an external source channels ratio of 1.95. Each sample was normalised to this purity (Table 1).

The vials used were of standard low potassium glass selected for weight similarity from a batch of 500. The background for each vial was monitored for 2-yr and was checked after each sample or standard filling. The vials were undercoated with white paint above the 18 ml level to improve photon collection and to give a precise counting geometry and then coated with black paint to reduce optical feedback between one photomultiplier and the other. Each vial was filled to within  $\pm 5$  mg of the normalised weight for benzene and scintillant (Table 1). The scintillation mixture in 1 ml of toluene added to 15 ml of sample benzene (13.1325 g) gave a scintillant concentration of 10 g l<sup>-1</sup> butyl PBD and 0.6 g l<sup>-1</sup> PBBO. An 'Agla' pipette with hypodermic needle was used to inject the sample through a special filling cap which was then replaced with a sealing cap incorporating discs of rubber, PTFE and pure tin. All samples were calculated using the Libby half life and 95% of the oxalic acid reference standard count rate representing AD 1950.

Each vial background was measured for successive 100-min intervals giving an accumulated count of approximately 100,000 for each vial. Each 100-min count was corrected for barometric



pressure variation and plotted to give a quality control distribution for that vial. Mean values of the vial distributions were compared in a single 'parent' distribution having a standard deviation equal to  $\pm 0.3\%$ . The points fell into an apparent normal distribution indicating that all the vials belonged to the same distribution thus allowing a single grand mean background value to be used. The values of the background and its precision including all errors and corrections were:  $9.289 \pm 0.078$  c.p.m.

Standards, samples and backgrounds were counted in sequence. Two oxalic reference standards were measured at any one time providing continuity and overlap during their regular replacement. Each measurement was corrected and plotted on a quality control chart, and a mean value determined for each individual oxalic distribution. As a check for unique vial efficiencies, high specific activity samples were used. On an observed count of not less than  $1.6 \times 10^7$  c.p.m. for each vial, no significant difference was obtained. The mean oxalic distributions were then compared and were found to lie within  $\pm 2$  standard deviations of a grand mean value, once more signifying that they probably belonged to a single distribution. The mean value of this distribution for the nett reference standard count rate used in the date calculation was:  $122.854 \pm 0.163$  c.p.m.

After synthesis each sample was left to stand at  $-10^\circ\text{C}$  for at least 1 month before being prepared for counting, to allow a 100-fold decrease in any remaining  $^{222}\text{Rn}$  contamination. At least 300,000 sample counts were accumulated over a minimum counting period of a fortnight. As in the case of standards and backgrounds, the barometric pressure and pulse-height variation were measured during each cycle and used for the correction of each result. Correction for weight changes was made after vial removal; any changes in cap weight being assessed and corrected for. All measurements were normalised to the value quoted in Table 1. The calculated dates were finally corrected for isotopic fractionation.

The 25-yr standard deviation quoted on all dates is a blanket precision covering all errors found to be significant in the experimental method. It includes not only the counting statistics and mass spectrometric correction normally quoted (equivalent in this case to less than  $\pm 17$  yr), but also the estimated errors due to all the corrections listed in Table 1. Some of the sources of error, if not corrected for, could cause a bias of up to 100 yr.

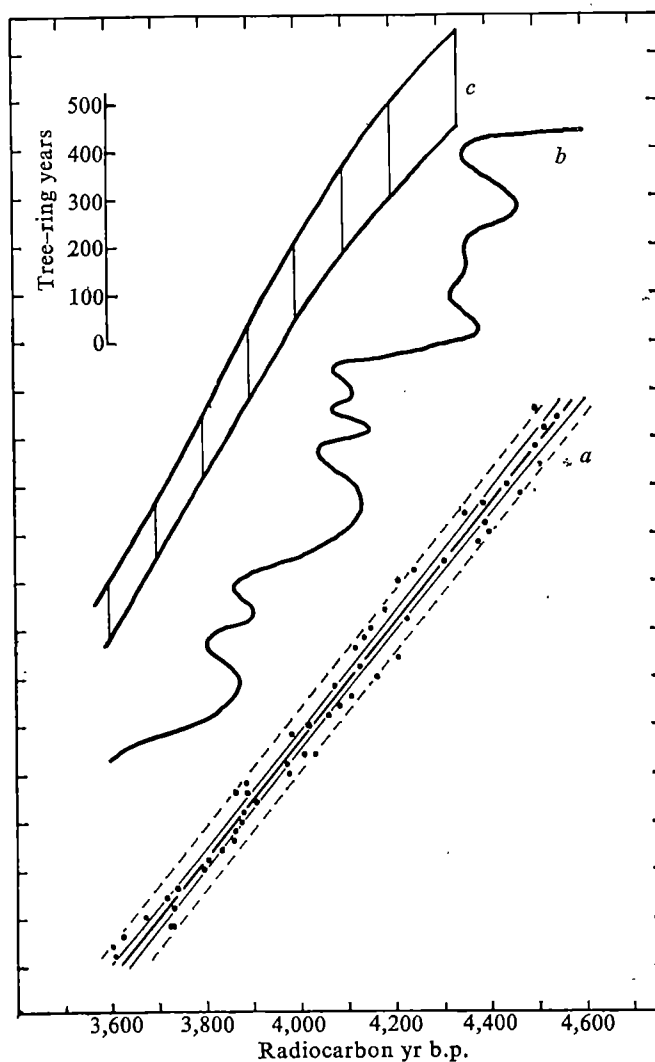
## Results and discussion

The results of the  $^{14}\text{C}$  measurements are given in Table 2, in yr before AD 1950. Some results for the contiguous series of samples are not yet available due to random selection of samples for

**Table 2** Radiocarbon dates b.p. for 20-yr increments of tree-ring dated Irish oak wood

Tree-ring age (yr)	$^{14}\text{C}$ date (b.p.)	Tree-ring age (yr)	$^{14}\text{C}$ date (b.p.)	Tree-ring age (yr)	$^{14}\text{C}$ date (b.p.)
0	3,604	400	3,969	800	4,240
20	3,600	420	4,007; 4,028	820	4,304
40	3,622	440	—	840	—
60	3,731; 3,722	460	3,981	860	4,376
80	3,669	480	4,017	880	4,399
100	3,728	500	4,059	900	4,390
120	3,714	520	4,081	920	4,347
140	3,737	540	4,107	940	4,387
160	—	560	4,072	960	4,466
180	3,792	580	4,161	980	4,438
200	3,801	600	4,125	1000	—
220	3,832	620	4,205	1020	—
240	3,856	640	4,115	1040	—
260	3,859	660	4,133	1060	4,499
280	3,875	680	4,146	1080	—
300	3,878	700	4,225	1100	4,517
320	3,906	720	4,179	1120	4,541
340	3,861; 3,884	740	—	1140	4,495
360	3,883	760	—	—	—
380	3,977	780	4,203	—	—

Tree-ring time scale is floating. 0 on this scale = 5,810 on the computer scale given in Pilcher *et al.*<sup>19</sup>.  $^{14}\text{C}$  dates are calculated on the 5,568 yr half life. 1  $\sigma$  precision on all dates is  $\pm 25$  yr.



**Fig. 1** Relationship between radiocarbon and tree-ring years. *a*, Relationship established using low-altitude oak from the North of Ireland; two standard deviation limits dashed, regression line drawn with curvilinear 95% confidence limits. *b*, Curve published by Suess<sup>7</sup> based on bristlecone pine wood. *c*, Historically derived calibration based on Egyptian material published by McKerrell<sup>18</sup>.

measurement, but no results have been deleted or ignored. The measurements are plotted on Fig. 1 together with the regression line through them. The regression line has a slope of 1.215, and a regression coefficient of 0.99. The *F* value for the regression is greater than 3,000 indicating a good straight line fit to the distribution. Also shown on Fig. 1 are sections of some previous calibration curves. Each is drawn on the same radiocarbon axis and on its own independent tree-ring axis. This is necessary because our tree-ring sequence is not yet tied to the present day.

The investigations carried out in preparation for these calibration measurements showed significant error sources in conventional  $^{14}\text{C}$  measurement techniques (G.W.P., unpublished). The results given here show that these error sources can be overcome and that the overall one standard deviation measurement precision can be reduced to less than 25 yr. The closeness of fit to a straight line indicates that there are no remaining significant random error sources not covered by the measurement precision. By increasing the sample counting time the precision could be reduced to the same level as other error sources giving an overall one standard deviation limit of  $\pm 12$  yr in 10,000, and similarly with a long count the limit of detection would be greater than 60,000 yr. If, as these results suggest, each radiocarbon age represents a unique calendrical age at least over a large part of the  $^{14}\text{C}$  time scale, then a very close approximation can be made to an absolute time scale.

The straight-line slope through our results lies within 1% of that derived by Damon *et al.*<sup>17</sup> and is close to the lines deduced by

Wendland and Donley<sup>8</sup> and by Clark<sup>13</sup> on the basis of the bristlecone pine results. The line based on Irish material does not show, within measurement limits, any of the wiggles predicted by Suess<sup>7</sup> for this period or retained by McKerrell<sup>18</sup> in his 50-yr averaging of the bristlecone pine data. This rules out, over the period investigated, the use of wiggle matching<sup>22</sup> as a dating technique. Suess (personal communication) has criticised our use of 20-yr blocks of tree-rings as he maintains that this will cause some smoothing of the wiggles. By using contiguous 20-yr blocks, however, no information is lost and the only way that short-term variations could produce a straight line calibration would be if they occurred as an exact harmonic of a 20-yr cycle over the whole 1,200 yr. This is inherently unlikely. Also, most radiocarbon samples represent greater than this time-span. Archaeological samples, which are very often of oak in European contexts, are normally charred branches or small timbers of at least 20 yr. Peat and sediment samples used for palaeoecological studies are normally of greater than 20-yr growth. The effect of smoothing due to deposition of recent carbon at the heartwood/sapwood transition has been circumvented by the use of wood cellulose as the dating material<sup>16</sup>. The only remaining non-random error which could explain the difference between the bristlecone pine and oak calibrations is the possibility<sup>16</sup> that persistent inversion layers along the Pacific coast of the USA may have caused deviations from the global average radiocarbon level. It is not clear whether this phenomenon could account for the short-term variations observed by Suess. Similarly, within the curvilinear limits of the regression there is no clear indication of the flat spots in the calibration predicted by Ottaway and Ottaway<sup>23</sup>.

Until the tree-ring sequences are tied to the present it will not be possible to assign calendar years to the tree-ring axis of our calibration curve. Without this it is not possible to answer questions of world uniformity of the atmospheric carbon reservoir<sup>24</sup>. If world-wide uniformity is assumed then the dendrochronological age of our calibration has been shown to be within 10 yr of the range 4,100–5,240 yr b.p. (J. C. Lerman, personal communication).

Even without absolute time control there are several important conclusions that can be drawn from these results. It is clear that for the radiocarbon age span 3,600–4,550 yr b.p. the calibration is smooth within the statistical uncertainty of  $\pm 3\%$  and is not significantly different from a straight line. As both the smoothness and the slope of the line are close to those statistically deduced from the bristlecone pine measurements it is reasonable to suggest that any calibration exhibiting marked deviations from this picture will ultimately be found in error. On the basis of these results it seems likely that the greater part of the calibration will tend to be smooth rather than 'wiggly'. This does not rule out, of course, the possibility of some short-term atmospheric radiocarbon variation such as that well documented for the seventeenth century AD. We cannot yet finally answer the question of whether there is a systematic difference between the bristlecone pine and Egyptian chronologies. The implication of the close agreement between the slopes of two tree-ring derived calibrations is that the deviant slope of the Egyptian calibration championed by McKerrell<sup>18</sup> is likely to be erroneous, indicating possible inaccuracies in the Egyptian

calendar or in  $^{14}\text{C}$  measurements for the period before 1800 BC.

Even within the short time span so far examined there are direct implications. For example, the floating calibration covers the disappearance of pine from Irish pollen diagrams for which a close bunching of radiocarbon dates has been observed<sup>25</sup>. This bunching can no longer be attributed to a 'calibration wiggle' and must, therefore, represent a synchronous event. Whether this same argument can be applied to problems outside the time range studied cannot yet be determined. If, however, most of the short-term variations disappear when a full calibration is completed, then it will ultimately reduce the uncertainty in the derivation of a true age estimate from a radiocarbon date. In fact, if it is assumed that a full calibration of the accuracy presented here is possible and if individual samples were measured with the ultimate precision of  $\pm 12$  yr, then it should be possible to produce a calibrated radiocarbon date whose 95% confidence limits fall within a bandwidth of 80 yr. This would restore radiocarbon dating to the position of the absolute dating method as was foreseen at its inception.

But the reality of the errors associated with radiocarbon dating must now be faced. Damon<sup>26</sup> and Clark<sup>13</sup> pointed out that the errors based on analysis of replicated bristlecone pine samples are greater than those covered by the quoted precision. Having demonstrated the linearity of the calibration it seems possible that the variation in the dates associated with the bristlecone pine samples was due to uncontrolled errors. Since the previous measurements involving calibration samples can be assumed to be among the most careful, even these errors may well be an underestimate of those associated with routine radiocarbon measurements.

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- DeVries, H. I. *Kon. Ned. Akad. Wetensch. Proc. B* **61**, 94–102 (1958).
- Willis, E. H., Tauber, H. & Münnich, K. O. *Am. J. Sci. Radiocarbon Suppl.* **2**, 1–4 (1960).
- Suess, H. E. *J. geophys. Res.* **70**, 5937–5952 (1965).
- Damon, P. E., Long, A. & Grey, D. C. *J. geophys. Res.* **71**, 1055–1063 (1966).
- Suess, H. E. *Proc. Symp. Radioactive Dating and Methods of Low-Level Counting, Monaco* 143–151 (IAEA, Vienna, 1967).
- Olsson, I. U. (ed.) *Radiocarbon Variations and Absolute Chronology* (Wiley, New York, 1970).
- Suess, H. E. in *Radiocarbon Variations and Absolute Chronology* (ed. Olsson, I. U.) 303–309 (Wiley, New York, 1970).
- Wendland, W. M. & Donley, D. L. *Earth planet. Sci. Lett.* **11**, 135–139 (1971).
- Clark, R. M. & Renfrew, C. *Archaeometry* **14**, 5–19 (1972).
- Michael, H. N. & Ralph, E. K. *Proc. 8th Int. Conf. Radiocarbon Dating, Lower Hutt City* **1**, 28–43 (1972).
- Birmingham, A. & Renfrew, C. *Antiquity* **46**, 151–153 (1972).
- Clark, R. M. *Antiquity* **49**, 251–266 (1975).
- LaMarche, V. C. & Harlan, T. P. *J. geophys. Res.* **78**, 8849–8858 (1973).
- Harkness, D. D. & Burleigh, R. *Archaeometry* **16**, 121–127 (1974).
- Cain, W. F. & Suess, H. E. *J. geophys. Res.* **81**, 3688–3694 (1976).
- Damon, P. E., Ferguson, C. W., Long, A. & Wallick, E. I. *Am. Antiquity* **39**, 350–366 (1974).
- McKerrell, H. in *Radiocarbon: Calibration and Prehistory* (ed. Watkins, T.) 47–100 (Edinburgh University Press, 1975).
- Pilcher, J. R., Baillie, M. G. L., Pearson, G. W. & Hillam, J. *New Phytol.* **79**, (in the press).
- Olson, E. A. & Broecker, W. S. *Trans. N. Y. Acad. Sci. Ser. II* **20**, 593 (1958).
- Baker, H. *Nature* **172**, 631–632 (1953).
- Suess, H. E. & Strahm, C. *Antiquity* **44**, 91–99 (1970).
- Ottaway, B. & Ottaway, J. H. *Nature* **250**, 407–408 (1974).
- Lerman, J. C., Mook, W. G. & Vogel, J. C. in *Radiocarbon Variations and Absolute Chronology* (ed. Olsson, I. U.) 275–301 (Wiley, New York, 1970).
- Smith, A. G. & Pilcher, J. R. *New Phytol.* **72**, 903–914 (1973).
- Damon, P. E. in *Radiocarbon Variations and Absolute Chronology* (ed. Olsson, I. U.) 571–593 (Wiley, New York, 1970).
- Callow, W. J., Baker, M. J. & Hassall, G. I. *Radiocarbon* **7**, 156–161 (1965).

## Purification of a positive regulatory subunit from phage SP01-modified RNA polymerase

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*A phage-induced subunit has been purified from phage SP01-modified Bacillus subtilis RNA polymerase. This subunit binds in vitro to RNA polymerase core from uninfected B. subtilis thereby inducing template-selective transcription and asymmetric synthesis of SP01 middle RNA.*

THE interaction of virus-coded proteins with the host's RNA polymerase is an important mechanism for the control of gene expression during bacteriophage development<sup>1,2</sup>. Purified RNA polymerase from *Bacillus subtilis* infected with phage SP01 or SP82 contains tightly associated, virus-coded proteins<sup>3–5</sup>. A significant

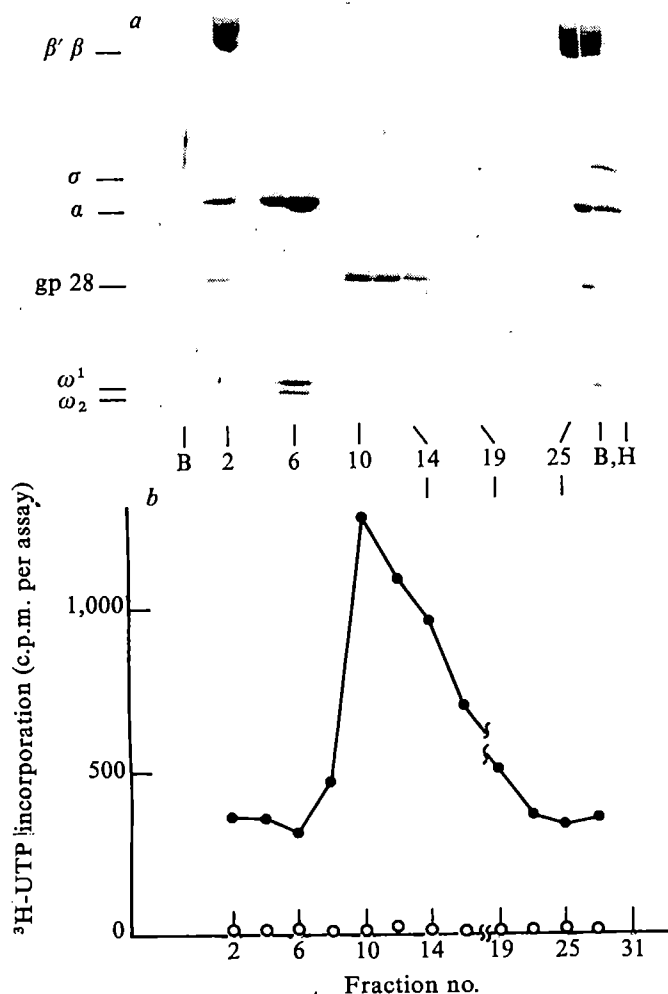
property of one particular modified RNA polymerase from phage SP01-infected bacteria is that it asymmetrically transcribes hydroxymethyluracil (hmU)-containing SP01 DNA to yield the positively regulated 'middle' SP01 RNA *in vitro*. In contrast, the unmodified host RNA polymerase transcribes essentially only SP01 'early' genes<sup>3-7</sup>. The modified RNA polymerase which we previously designated as B-P is composed of the subunits of polymerase core together with two virus-coded subunits,  $\nu^{28}$  and  $\nu^{13}$  (ref. 3). (The quantity of  $\nu^{13}$  varies from preparation to preparation.) At least one of the two virus-coded subunits is responsible for the selective, rapid initiation of SP01 middle transcription and functions at the level of DNA binding<sup>8</sup>. We have previously reported<sup>9</sup> the identification of a protein from a partially purified extract of SP01-infected bacteria which binds to host RNA polymerase core and converts it to the modified form. This protein has the physical properties of  $\nu^{28}$ . The isoelectric point (unpublished observations) and molecular weight of  $\nu^{28}$  identify it as identical with the peptide IV of Pero *et al.*<sup>4</sup>, which has been shown<sup>10</sup> to be the product of SP01 gene 28. The product of SP01 gene 28 controls 'middle' viral gene expression<sup>11</sup>. Thus,  $\nu^{28}$  = peptide IV = gene product 28 (gp28). We report here the purification of gp28 from RNA polymerase and show that gp28 directs the transcription of SP01 'middle' genes by *B. subtilis* RNA polymerase core enzyme.

### Purification of regulatory subunit

The RNA polymerase which we used as a source of gp28 was purified from *B. subtilis* which had been infected for 10 min with

phage SP01 *sus* F4 (gene 33)-*sus* F14 (gene 34). Genes 33 and 34 code for proteins that regulate late transcription and bind to host RNA polymerase<sup>7,11-13</sup>. The phage-modified RNA polymerase from *sus* F4-*sus* F14-infected bacteria is free of gp33 and gp34. It is composed of the RNA polymerase core subunits ( $\beta$ ,  $\beta'$ ,  $\alpha$ ,  $\omega^1$ ,  $\omega^2$ ) and of the virus-coded subunit gp28 (data not shown).

When RNA polymerase is dissociated with 6 M urea and chromatographed on phosphocellulose in the presence of 6 M urea, the subunits of the polymerase are separated. Figure 1a shows a gel electropherogram of the fractions obtained. The  $\alpha$ ,  $\omega^1$  and  $\omega^2$  subunits (fractions 4-8) do not bind to the phosphocellulose. Elution of gp28 is reproducibly sufficiently delayed to separate it from the  $\alpha$ ,  $\omega^1$  and  $\omega^2$  subunits (fractions 10-19). The  $\beta'$  and  $\beta$  subunits bind to phosphocellulose and are eluted at higher salt concentration in 6 M urea (0.12 M and 0.27 M KCl, respectively). The pattern of subunit fractionation is similar to that previously described<sup>14</sup>. But, the RNA polymerase from *sus* F4-*sus* F14-infected *B. subtilis* lacks subunit  $\nu^{13}$  which would otherwise elute with gp28 and, as a result of this, gp28 is eluted in electrophoretically pure form. The fractionation of the  $\beta$ ,  $\beta'$ ,  $\alpha$ ,  $\omega^1$  and  $\omega^2$  subunits is also similar to that reported by Tjian and Pero<sup>13</sup>. Comparison of Fig. 1a and b shows a direct correlation between the activity which stimulates RNA polymerase core and the presence of gp28. The stimulating activity is manifested only in the presence of hmU-containing SP01 DNA and not with thymine-containing  $\phi$ 29 DNA. This template selectivity is also a property of the phage-modified RNA polymerase B-P<sup>3,8</sup>. The activity of gp28 can be assayed without prior removal of urea. Either gp28 is not denatured in the conditions of the phosphocellulose chromato-



**Fig. 1** Phosphocellulose chromatography of urea-treated RNA polymerase from *B. subtilis* infected for 10 min with *sus* F4-*sus* F14 SP01 phage. RNA polymerase (1.2 mg, purified according to ref. 3) was dialysed against buffer G (0.01 M Tris-HCl, pH 8, 0.1 mM EDTA, 0.01 M dithiothreitol) containing 0.05 M KCl and 6 M urea (freshly deionised) and was subsequently applied to a column of phosphocellulose (1.4 cm  $\times$  0.7 cm i.d., column volume 2 ml, rate of application 2 ml per h) pre-washed with 200 ml of the same buffer. The polymerase was allowed to equilibrate with the phosphocellulose for 30 min. The column was then washed with the same buffer at a rate of 2 ml per h, and 1 ml fractions were collected into tubes containing 0.25 ml of 50% (v/v) glycerol in buffer G plus 0.05 M KCl. *a*, Polyacrylamide slab gel electrophoresis. 100  $\mu$ l aliquots of selected fractions from the phosphocellulose column were concentrated by precipitation on ice in 10% (w/v) trichloroacetic acid. The precipitates were collected by centrifugation, washed with cold 1 M HCl and again collected by centrifugation. The samples were dried under vacuum and dissolved in the electrophoresis sample buffer which contained 3% (w/v) sodium dodecylsulphate to aid in solubilising the samples. The samples were electrophoresed in denaturing conditions (0.1% SDS) in a polyacrylamide slab gradient gel (7-15% (w/v) acrylamide) run according to Laemmli<sup>17</sup>. Marker samples were treated in an identical manner. The gels were fixed and stained in 0.2% Coomassie brilliant blue in methanol-acetic acid-water (4.5:1:4.5) and destained in methanol-acetic acid-water (10:7.5:82.5). The following samples are shown: B, 3  $\mu$ g of RNA polymerase from *B. subtilis* infected for 10 min with *sus* F4-F14 SP01 phage; numbered fractions from the phosphocellulose column; H, 3  $\mu$ g of RNA polymerase from uninfected *B. subtilis*. *b*, Assay of fractions for their ability to stimulate the activity of RNA polymerase core from uninfected *B. subtilis*. 10  $\mu$ l aliquots of fractions from the phosphocellulose column were added on ice to 0.4  $\mu$ g of RNA polymerase core in 25  $\mu$ l of TGB (0.01 M Tris-HCl pH 8, 0.1 mM EDTA, 15% (v/v) glycerol, 400  $\mu$ g ml<sup>-1</sup> bovine serum albumin). Then 4  $\mu$ g of DNA was added in 20  $\mu$ l of 0.01 M Tris-HCl pH 7.5 and 0.001 M EDTA. Each sample was then made up to 90  $\mu$ l by addition of reaction components to give the following concentration: 0.11 M Tris-HCl pH 8, 0.11 mM each of EDTA and dithiothreitol, 0.88 mM spermidine chloride, 1.1 mM each of ATP, GTP, CTP, and 0.13 mM  $^3\text{H}$ -UTP (10.3 c.p.m. pmol<sup>-1</sup>). After incubation for 10 min at 30  $^{\circ}\text{C}$ , 10  $\mu$ l of 0.1 M MgCl<sub>2</sub> was added to start the reaction (the final urea concentration was 0.48 M). After 5 min the reaction was stopped and incorporation of  $^3\text{H}$ -UTP into acid insoluble material was measured as described elsewhere<sup>3</sup>.  $^3\text{H}$ -UTP incorporation was measured using either SP01 DNA ( $\bullet$ ) or  $\phi$ 29 DNA ( $\circ$ ) as template. In the presence of RNA polymerase core and with the column fractions replaced by 10  $\mu$ l of the equivalent buffer, incorporation was 280 c.p.m. with SP01 DNA and less than 20 c.p.m. with the preparation of  $\phi$ 29 DNA used here. Using the same conditions, 0.4  $\mu$ g *B. subtilis* holoenzyme gave 1,942 c.p.m. incorporation in the presence of SP01 DNA and 982 c.p.m. in the presence of  $\phi$ 29 DNA. (Preparations of  $\phi$ 29 DNA used by us previously gave appreciably more template activity with *B. subtilis* RNA polymerase core<sup>3</sup>.)

graphy, or, more probably, it renatures rapidly in the presence of polymerase core at the lower urea concentration (0.48 M) of the assay.

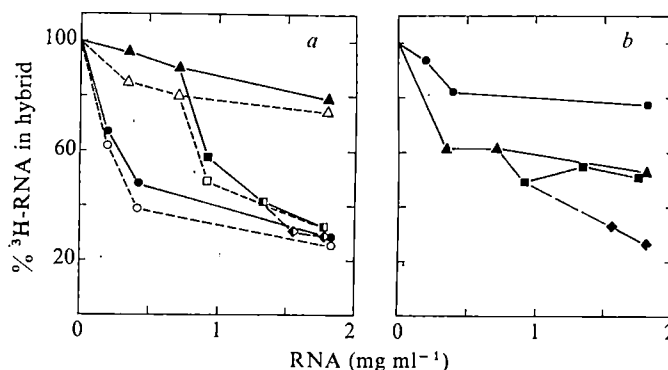
Fractions containing gp28 were combined and their glycerol content was increased to 25% (v/v). The gp28 was concentrated and the urea was removed by passing the combined fractions over a DEAE-cellulose column (1.8 cm  $\times$  0.4 cm i.d., 1 ml column volume) and subsequently washing the column with urea-free buffer (buffer G with 0.04 M  $\text{NH}_4\text{Cl}$  and 25% (v/v) glycerol added). A gradient of 0.04–1.0 M  $\text{NH}_4\text{Cl}$  in the same buffer was applied to the column and gp28 was eluted at 0.2 M  $\text{NH}_4\text{Cl}$ .

### Properties of regulatory subunit

The activity and template selectivity of RNA polymerase reconstituted from purified gp28 and *B. subtilis* core enzyme and of the phage-modified RNA polymerase from *sus* F4-*sus* F14 phage-infected bacteria are compared in Table 1. The quantity of gp28 added to the RNA polymerase is saturating with respect to polymerising activity on SP01 DNA. The template selectivity of the gp28 is shown by the more than fourfold stimulation of RNA polymerase core in the presence of hmU-containing SP01 DNA (line 1) and by the lack of activity of thymine-containing  $\phi$ 29 DNA (line 2). The same selectivity is observed with the RNA polymerase from *sus* F4-*sus* F14-infected bacteria (lines 3 and 4) and with gp28-containing RNA polymerase from *B. subtilis* infected for 10 min with wild type SP01 (refs 3, 8). The phage-modified polymerase from *sus* F4-*sus* F14-infected bacteria seems to be purified with near-saturating amounts of gp28 since it can be stimulated only 1.2-fold by the addition of purified gp28 (line 3). But, the maximum activity of the phage-modified RNA polymerase in the presence of saturating amounts of gp28 remains significantly less than that obtained with the combination of *B. subtilis* RNA polymerase core and gp28 (compare lines 1 and 3). It is possible that this difference is entirely due to effects introduced during the purification of these enzymes (for example, our preparations of enzyme from phage-infected cells may consistently contain a significant fraction of inactive material). But, the difference of enzyme activity may also reflect a modification of polymerase core after phage infection which affects its interaction with gp28. Such modifications are known to occur after infection with other phages (see ref. 2 for review) but have not yet been detected after phage SP01 infection.

When gp28 is combined with host RNA polymerase core, the reconstructed RNA polymerase has the ability to transcribe 'middle' SP01 genes asymmetrically *in vitro* as shown in Figs 2 and 3.  $^3\text{H}$ -RNA was synthesised for 10 min *in vitro* with excess native SP01 DNA in the presence of 0.04 M  $\text{NH}_4\text{Cl}$  and 0.16 M KCl and was analysed by hybridisation-competition with unlabelled RNA extracted from phage SP01-infected *B. subtilis*. The rationale of this analysis was described previously<sup>15</sup>. Figure 2a compares the

competition patterns of  $^3\text{H}$ -RNA made *in vitro* by *B. subtilis* polymerase core with added purified gp28 and of  $^3\text{H}$ -RNA made by the phage-modified RNA polymerase isolated with bound gp28. The similarity of these two *in vitro* synthesised RNA samples is obvious. Both are composed mainly of middle RNA transcripts as judged by the following criteria: (1) poor competition by *in vivo* early RNA; (2) maximum competition when *in vivo* middle RNA is



**Fig. 2** Hybridisation-competition analysis of SP01  $^3\text{H}$ -RNA synthesised *in vitro* with *B. subtilis* RNA polymerase core in absence or presence of gp28.  $^3\text{H}$ -RNA was prepared by first combining the following components in sequence on ice in 240  $\mu\text{l}$ : 50  $\mu\text{l}$  of gp28 in buffer G containing 0.2 M  $\text{NH}_4\text{Cl}$  and 25% (v/v) glycerol or 50  $\mu\text{l}$  of the same solution without gp28, 1  $\mu\text{g}$  of *B. subtilis* RNA polymerase core, 10  $\mu\text{g}$  of SP01 DNA, 1 mM each of ATP, GTP and CTP, 0.12 mM  $^3\text{H}$ -UTP (560 c.p.m.  $\text{pmol}^{-1}$ ), 0.1 M Tris-HCl, pH 8, 0.1 mM each of EDTA and dithiothreitol, 0.8 mM spermidine, 0.16 M KCl. The sample was incubated for 5 min at 30  $^{\circ}\text{C}$ , and then was made 0.01 M  $\text{MgCl}_2$  by addition of 10  $\mu\text{l}$  of 0.25 M  $\text{MgCl}_2$ . After 10 min, 25  $\mu\text{l}$  of 0.2 M EDTA and 0.2% SDS was added to stop the reaction. Synthesis of  $^3\text{H}$ -RNA by RNA polymerase from *B. subtilis* infected for 10 min with *sus* F4-*sus* F14 double mutant of SP01 phage was essentially the same except for the following changes: the final volume was 200  $\mu\text{l}$  containing 7.2  $\mu\text{g}$  SP01 DNA, 0.2 M KCl, and 0.1 mM  $^3\text{H}$ -UTP (750 c.p.m.  $\text{pmol}^{-1}$ ). The  $^3\text{H}$ -RNA was extracted with phenol and prepared for analysis as described elsewhere<sup>16</sup>. The conditions of RNA synthesis used here differ from those which we used previously<sup>3,6</sup> and resemble those of Losick and Pero<sup>18</sup>. We have previously assayed asymmetry of transcription at lower ionic strength, while limiting RNA synthesis to promoter-proximal segments of RNA<sup>3,6</sup>. Losick and Pero<sup>18</sup> showed *in vitro* asymmetrical transcription by modified polymerases at higher ionic strength during relatively long periods of synthesis. We compared both procedures with the phage-modified RNA polymerase B-P and found similar degrees of asymmetry as measured by RNA-RNA duplex formation. We used the conditions specified here because they yield more RNA for a given quantity of gp28. Hybridisation of the  $^3\text{H}$ -RNA (0.02  $\mu\text{g ml}^{-1}$ ) to SP01 DNA (10  $\mu\text{g ml}^{-1}$ ) was competed against by unlabelled RNA from SP01 infected *B. subtilis*. The aim of the competition experiments is to distinguish early, middle and late viral RNA together with their symmetrical antimesenger counterparts. Each of these three classes of messages is composed of subclasses (see ref. 16). Early (*e* and *em*) RNA is synthesised when infection is in the presence of chloramphenicol (CAM). We collected this RNA 12 min after infection at 37  $^{\circ}\text{C}$ . RNA synthesised after infection with SP01 *sus* F14 (gene 33) phage and collected 12 min after infection contains middle RNA species (*em*, *m* and *m*<sub>1</sub>). Late RNA (*m*<sub>2</sub> and *l*) is collected 28 min after infection with wild type phage. (The mixed competitor sequence used here allows us to neglect questions of transcriptional shut-off *in vivo*.)  $\Delta$ ,  $\blacktriangle$ , Early RNA;  $\circ$ ,  $\bullet$ , middle RNA;  $\square$ ,  $\blacksquare$ , mixed competition of 0.72 mg  $\text{ml}^{-1}$  early RNA with increasing concentrations of middle RNA;  $\diamond$ ,  $\blacklozenge$ , mixed competition of 0.72 mg  $\text{ml}^{-1}$  early RNA, 0.61 mg  $\text{ml}^{-1}$  middle RNA and increasing concentrations of late RNA. *a*,  $^3\text{H}$ -RNA synthesised with RNA polymerase core from uninfected *B. subtilis* with added gp28 (solid line, closed symbols) and  $^3\text{H}$ -RNA synthesised with phage-modified RNA polymerase from *sus* F4-*sus* F14 SP01 infected *B. subtilis* (subunit composition  $\beta$ ,  $\beta'$ ,  $\alpha$ , gp28,  $\omega^1$ ,  $\omega^2$ ; dashed line, open symbols). The input of  $^3\text{H}$ -RNA made with the reconstituted RNA polymerase was 927 c.p.m., and hybridisation in the absence of competing RNA was 972 c.p.m. The radioactivity bound to the filter in the absence of DNA was 14 c.p.m. The input of  $^3\text{H}$ -RNA made with the phage-modified RNA polymerase was 881 c.p.m., and hybridisation in the absence of competing RNA was 579 c.p.m. The radioactivity bound to the filter in the absence of DNA was 30 c.p.m. *b*,  $^3\text{H}$ -RNA synthesised with RNA polymerase core from uninfected *B. subtilis*. Input radioactivity was 555 c.p.m. per assay and hybridisation in the absence of competing RNA was 494 c.p.m. The radioactivity bound to the filter in the absence of DNA was 14 c.p.m.

**Table 1** Activity and template selectivity of RNA polymerase reconstituted from purified gp28 and *B. subtilis* RNA polymerase core

RNA polymerase	DNA	c.p.m.	
		(-)gp28	(+)gp28
1 <i>B. subtilis</i> core	SP01	811	3,458
2 <i>B. subtilis</i> core	$\phi$ 29	58	99
3 <i>sus</i> F4- <i>sus</i> F14	SP01	1,976	2,371
4 <i>sus</i> F4- <i>sus</i> F14	$\phi$ 29	93	—

The following components were first combined in sequence on ice: 5  $\mu\text{l}$  of gp28 in buffer G with 25% (v/v) glycerol and 0.2 M  $\text{NH}_4\text{Cl}$  or 5  $\mu\text{l}$  of buffer alone, 0.1  $\mu\text{g}$  RNA polymerase in 25  $\mu\text{l}$  of TGB, 1  $\mu\text{g}$  of DNA in 10  $\mu\text{l}$  0.01 M Tris-HCl, pH 7.5, 0.001 M EDTA, 50  $\mu\text{l}$  of reaction components to give 90  $\mu\text{l}$  containing 0.11 M Tris-HCl, pH 8, 0.11 mM each of EDTA and dithiothreitol, 0.88 mM spermidine chloride, 1.1 mM each of ATP, GTP, CTP, and 0.13 mM  $^3\text{H}$ -UTP (100 c.p.m.  $\text{pmol}^{-1}$ ). After a 5 min incubation at 30  $^{\circ}\text{C}$ , the reaction was started by adding 10  $\mu\text{l}$  of 0.1 M  $\text{MgCl}_2$ . After a further 5 min the reaction was stopped and incorporation of  $^3\text{H}$ -UTP into acid insoluble material was measured as described previously<sup>3</sup>. Incorporation for 0.1  $\mu\text{g}$  RNA polymerase holoenzyme from uninfected *B. subtilis* was 4,501 c.p.m. in the presence of SP01 DNA and 3,887 c.p.m. in the presence of  $\phi$ 29 DNA. Incorporation in the absence of RNA polymerase core and in the presence of gp28 was 24 c.p.m.

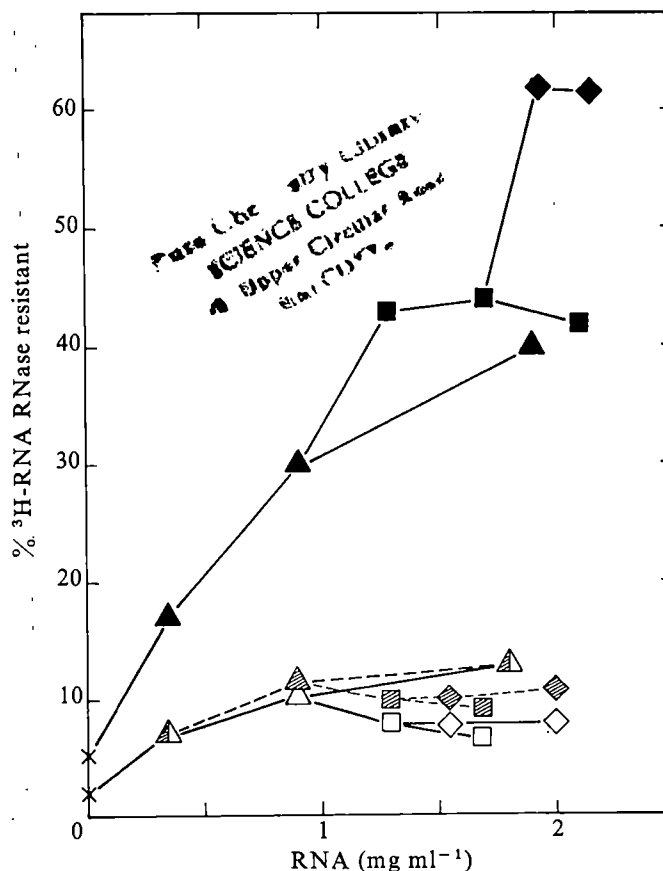
added to early RNA; and (3) poor competition by added *in vivo* late RNA. The hybridisation-competition pattern of RNA synthesised *in vitro* with *B. subtilis* RNA polymerase core alone (Fig. 2b) is distinctly different in that all the *in vivo* RNA's compete to some extent, but poorly. The qualitative effect that gp28 has on the composition of  $^3\text{H}$ -RNA made *in vitro* is most strikingly evident when the asymmetry of the *in vitro*-synthesised RNA is assayed in terms of the ability to form RNase-resistant RNA-RNA duplexes with early, middle and late *in vivo* RNA. Figure 3 shows that the  $^3\text{H}$ -RNA made by RNA polymerase core alone is symmetrical (see further comments below). In contrast, the  $^3\text{H}$ -RNA made with RNA polymerase core in the presence of added gp28 is relatively asymmetrical. More specifically, the mixed competitor assays for hybridisation-competition and the mixed RNA assays for RNA-RNA duplex formation (Figs 2 and 3) provide the following conclusions: that while the minor component of transcription from early genes is relatively symmetrical, the major component of middle RNA is almost perfectly asymmetrical. The pattern of RNA-RNA duplex formation by the RNA made with RNA polymerase from *sus* F4-*sus* F14 SP01 phage infected *B. subtilis* is identical to that for RNA made with RNA polymerase core and gp28.

Some comment on the symmetry of the SP01 RNA synthesised with *B. subtilis* core enzyme is appropriate. The data shown here imply that a slight excess of anti-messenger is synthesised (57% duplex at the highest concentration of all RNAs used, after subtracting background). This is approximately the value previously obtained in somewhat different conditions of transcription<sup>3</sup>. Approximately equal hybridisation of *in vitro* RNA to complementary DNA strands is often interpreted as indicating random transcription of helical SP01 DNA by core enzyme. While asymmetry of RNA synthesis is an excellent indication of selectivity of transcription, the reverse inference is not valid. There is as yet no clear evidence that initiation by any prokaryotic RNA polymerase core enzyme on any continuous natural DNA template is random (that is, able to use any pyrimidine nucleotide in DNA to signal initiation). Our finding of a slight excess of antimessenger synthesised by RNA polymerase core is consistent with at least partially selective transcription. This selectivity clearly generates a collection of transcripts that are very different from *in vivo* RNA and from the transcripts generated by the fidelity of the complete transcriptases. The observed selectivity of transcription by *B. subtilis* RNA polymerase core could arise at the level of initiation or at termination.

## Discussion

Thus, gp28, through its binding to RNA polymerase, is responsible for directing the asymmetrical synthesis of middle SP01 RNA *in vitro*. We surmise that gp28 confers on RNA polymerase core the template-specific ability to form rapid initiation complexes with SP01 DNA which we previously demonstrated to be a property of the phage-modified RNA polymerase<sup>8</sup>. How do these properties of RNA polymerase relate to the regulation of phage SP01 transcription? Fujita *et al.*<sup>11</sup> presented a model for the regulation of SP01 transcription based on an analysis of RNA synthesis during phage development<sup>16</sup> and on the properties of SP01 mutations which affect viral transcription. The model, in its simplest form, involved a positive effector for the initiation of middle SP01 transcription, a positive effector composed of two proteins, for late SP01 RNA synthesis and a single negative element for the repression of a class of middle SP01 RNA. The products of two genes (33 and 34) which regulate the appearance of late classes of SP01 RNA have been shown to be RNA polymerase-binding proteins<sup>12</sup>. When added to *B. subtilis* RNA polymerase core, these two subunits act synergistically to promote late SP01 RNA synthesis *in vitro*<sup>13</sup>. These results combined with our observations and those of Fox *et al.*<sup>10</sup>, identify the three RNA-polymerase binding proteins as two of the positive effectors proposed by Fujita *et al.*<sup>11</sup>.

Although virus-coded proteins which bind to host RNA polymerase have been described<sup>1,2</sup>, only those regulating SP01



**Fig. 3** RNA-RNA duplexes between *in vivo* RNA and  $^3\text{H}$ -RNA synthesised *in vitro* with *B. subtilis* RNA polymerase core in the absence or presence of gp28 and  $^3\text{H}$ -RNA synthesised with phage-modified RNA polymerase from *sus* F4-*sus* F14 SP01 infected *B. subtilis*. The  $^3\text{H}$ -RNA analysed by hybridisation-competition in Fig. 2a and b was assayed for the formation of RNA-RNA duplexes with *in vivo* RNA from SP01 infected bacteria as described elsewhere<sup>6,19</sup>. Each sample contained approximately  $0.02 \mu\text{g ml}^{-1}$   $^3\text{H}$ -RNA. For  $^3\text{H}$ -RNA synthesised by modified RNA polymerase from *B. subtilis* infected with SP01 *sus* F4-*sus* F14 (cross-hatched symbols; dashed line) and by polymerase core in the absence (filled symbols) or the presence of gp28 (open symbols) input radioactivity was 881 c.p.m., 555 c.p.m. and 927 c.p.m., respectively. The  $^3\text{H}$ -RNA was annealed to increasing concentrations of early RNA ( $\Delta$ , cross-hatched triangle,  $\blacktriangle$ ), 0.90  $\text{mg ml}^{-1}$  early RNA and increasing concentration of middle RNA ( $\square$ , cross-hatched square,  $\blacksquare$ ), and 0.90  $\text{mg ml}^{-1}$  early RNA, 0.80  $\text{mg ml}^{-1}$  middle RNA with increasing concentrations of late RNA ( $\diamond$ , cross-hatched diamond,  $\blacklozenge$ ).

development have up till now been amenable to *in vitro* analysis of selective transcription. The availability of these SP01-coded subunits should allow a closer study of the means by which they exert their regulatory influence. It may now be possible to study, *in vitro*, the dynamic interaction of these effectors with bacterial  $\sigma$  factor and with RNA polymerase core and, in that way, to understand whether these interactions alone can generate the major transitions of transcription that characterise gene expression during phage SP01 development. Models generated by such an investigation may relate to other developmental processes, at least in prokaryotes.

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1. Stevens, A. *Proc. natn. Acad. Sci. U.S.A.* **69**, 603-609 (1972).
2. Rabussay, D. & Geiduschek, E. P. in *Comprehensive Virology* **8**, (eds Fraenkel-Conrat, H. & Wagner, R.) 1-196 (Plenum, New York and London, 1977).
3. Duffy, J. J. & Geiduschek, E. P. *J. biol. Chem.* **250**, 4530-4541 (1975).
4. Pero, J., Nelson, J. & Fox, T. D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1589-1593 (1975).
5. Whiteley, H. R., Spiegelman, G. B., Lawrie, J. M. & Hiatt, W. R. in *RNA Polymerase* (eds Chamberlin, M. & Losick, R.) 587-600 (Cold Spring Harbor Laboratory, New York, 1976).
6. Duffy, J. J. & Geiduschek, E. P. *FEBS Lett.* **34**, 172-174 (1973).
7. Pero, J., Tjian, R., Nelson, J. & Fox, T. D. *Nature* **257**, 248-251 (1975).



8. Duffy, J. J. & Geiduschek, E. P. *Cell* **8**, 595-604 (1976).
9. Duffy, J. J., Petrusek, R. L. & Geiduschek, E. P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2366-2370 (1975).
10. Fox, T. D., Losick, R. & Pero, J. *J. molec. Biol.* **101**, 427-433 (1976).
11. Fujita, D. J., Ohlsson-Wilhelm, B. M. & Geiduschek, E. P. *J. molec. Biol.* **57**, 301-317 (1971).
12. Fox, T. D. *Nature* **262**, 748-753 (1976).
13. Tjian, R. & Pero, J. *Nature* **262**, 753-757 (1976).
14. Petrusek, R. L., Duffy, J. J. & Geiduschek, E. P. in *RNA Polymerase* (eds Chamberlin, M. & Losick, R.) 567-585 (Cold Spring Harbor Laboratory, New York, 1976).
15. Grau, O., Ohlsson-Wilhelm, B. M. & Geiduschek, E. P. *Cold Spring Harbor Symp. quant. Biol.* **35**, 221-226 (1970).
16. Gage, L. P. & Geiduschek, E. P. *J. molec. Biol.* **57**, 279-300 (1971).
17. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
18. Losick, R. & Pero, J. in *RNA Polymerase* (eds Chamberlin, M. & Losick, R.) 227-246 (Cold Spring Harbor Laboratory, New York, 1976).
19. Grau, O. & Geiduschek, E. P. in *Lepetit Colloquia on Biology and Medicine: RNA Polymerase and Transcription* (ed. Silvestri, L.) 190-203 (North-Holland, Amsterdam, 1969).

# Endogenous inhibitor of prostaglandin synthetase

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*Mammalian serum and plasma contain an endogenous inhibitor of prostaglandin synthetase (EIPS). Human plasma fractions rich in EIPS show anti-inflammatory activity in vivo. In rats, glucocorticoids raise EIPS activity of plasma and serum. These findings suggest the existence of a natural mechanism of controlling prostaglandin synthesis, possibly related to corticosteroid action.*

PROSTAGLANDINS (PGs) can elicit or intensify various unpleasant effects such as vomiting, diarrhoea, cough, pain and inflammation. Although PGs may well have an underlying defensive function, their production can at times be excessive and harmful<sup>1</sup>. There seems, therefore, to be a need to control PG levels in tissues. It is known that these levels can be controlled through the availability of substrate for PG synthetase or the activity of enzymes metabolising prostaglandins. We describe here a new, direct endogenous mechanism of inhibiting PG synthesis that could also contribute to the control of PG levels. This was brought to light by the observation (by S.A.S.) that human serum potentially inhibited the conversion of arachidonic acid into PGE<sub>2</sub> and PGF<sub>2α</sub> by homogenate of bull seminal vesicles (BSV). Serum and plasma of other mammals also possess this property. In human plasma, this property was associated with a particular fraction, the activity of which could be increased by further fractionation. This and other evidence indicated the presence of an endogenous inhibitor of prostaglandin synthetase (EIPS). We outline below experiments on the occurrence of EIPS, on its biochemical and pharmacological properties and on its possible relationship to the mechanism of action of anti-inflammatory steroids.

## Inhibition of PG synthesis by mammalian serum and plasma

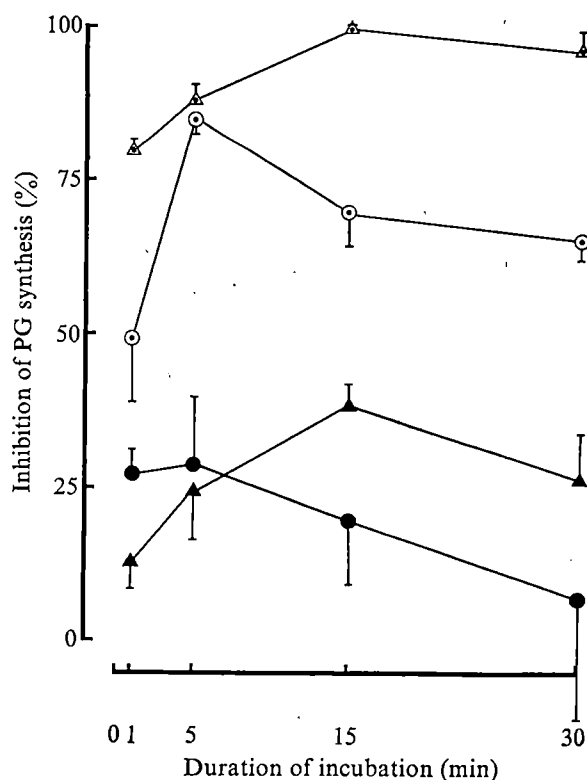
Two methods were used for measuring the extent of PG synthesis *in vitro*. First, arachidonic acid was incubated with bovine seminal vesicle homogenate and the PGs formed were either estimated as total PG-like activity on rat or hamster stomach fundus or separated by thin-layer chromatography (TLC) and estimated as PGE<sub>2</sub> on these tissues or as PGF<sub>2α</sub> on rat colon, as previously described<sup>2</sup>. Second, tritiated arachidonic acid was incubated with bovine seminal vesicle homogenate, the PGE<sub>2</sub> and PGF<sub>2α</sub> formed were separated by TLC and estimated by liquid scintillation spectrometry. Further details of both methods are given in Tables 1 and 2.

Table 1 illustrates the concentration-response relationships for inhibition of total PG synthesis by several vertebrate sera. Except chicken, which was inactive, these sera, at concentrations of 25% v/v or less, inhibited PG production by 70% or

more. These effects were concentration-related ( $P < 0.05$  for slopes of concentration-response lines). The mean values with standard error for percentage concentration (v/v) to inhibit PG production by 50% (IC<sub>50</sub>) of the sera tested were: rat,  $1.11 \pm 0.21\%$ ; human,  $1.37 \pm 0.55\%$ ; dog,  $2.96 \pm 0.3\%$ ; rabbit,  $7.50 \pm 1.35\%$ ; mare,  $9.30 \pm 0.41\%$ .

In a few experiments, sera of guinea pig, ox and foetal calf were also tested. Guinea pig and ox sera inhibited PG synthesis at dilutions comparable with those in Table 1, but foetal calf serum was ineffective. Inhibitory activity was also found in time-expired human citrated plasma from a blood bank and in fresh rat plasma containing  $0.2 \text{ mg ml}^{-1}$  of disodium EDTA. When incubated in the standard test conditions with BSV homogenate, the IC<sub>50</sub> values of the citrated human plasma and fresh rat plasma were  $0.78 \pm 0.06\%$  v/v and  $1.74 \pm 0.19\%$  v/v respectively.

**Fig. 1** Time course of inhibition by human serum or aspirin of PG synthesis by bovine seminal vesicle homogenate; ●, 0.5% serum v/v; ○, 5.0% serum v/v; ▲, 0.625 mM aspirin; and △, 3.13 mM aspirin. Incubations were conducted for 1, 5, 15 and 30 min. Values are the means of three experiments. Other details as in Table 1.



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Table 1 Inhibition of prostaglandin synthesis by sera

Serum concentration (%v/v)	Rat	Human	Inhibition of total PG production (%) Dog	Rabbit	Mare	Chicken
0.05	NT	13.7±5.8	NT	NT	NT	NT
0.5	27.7±4.91	34.7±13.7	14.0±4.5	2.1±1.2	15.0±9.0	NA
2.5	65.6±8.56	NT	NT	NT	NT	NT
5.0	93.3±2.14	88.7±2.2	61.0±2.1	16.8±9.0	25.0±3.0	NA
25.0	NT	90.0±3.5	97.7±0.38	91.5±0.9	91.0±1.0	NA

Inhibition values are mean  $\pm$  s.e.m. NA, not active; NT, not tested. Inhibition, by pooled serum from each species, of PG production was measured in a standard assay mixture (2 ml), containing 50 mM phosphate buffer, 0.25 ml Na<sub>2</sub>EDTA, 1.3 mM reduced glutathione, 0.5 ml bovine seminal vesicle homogenate, and an appropriate volume of the test serum or of isotonic saline as control<sup>2</sup>. The reaction was started by the addition of sodium arachidonate (final concentration 61  $\mu$ M) and the tubes aerated at 37 °C with gentle shaking. After 15 min, 2 ml of 0.2 M citric acid and 16 ml ethylacetate were added to stop the reaction. After mixing and centrifugation for 5 min at 600g, 10 ml of the ethylacetate layer was evaporated to dryness, the residue was redissolved in Krebs solution and PG-like activity of the samples assayed on rat or hamster<sup>3</sup> fundus, using PGE<sub>2</sub> as reference.

In some experiments, the PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  produced by bovine seminal vesicle homogenate in the presence of various concentrations of human serum and in its absence, were separated by TLC using the AI solvent system, and assayed on rat fundus and rat colon respectively. Human serum, at 0.5, 5 and 25% v/v, inhibited production of PGE<sub>2</sub> by 29.7±14.9, 88.7±5.8 and 96.5±3.2% respectively. The serum at these concentrations inhibited PGF<sub>2 $\alpha$</sub>  production by 58.0±17.1, 78.7±3.9 and 73.7±2.4% respectively. That the slope of the concentration-response line for PGF<sub>2 $\alpha$</sub>  was not significant may be attributed to the high standard error of the estimate at 0.5% serum. In the experiments described below with labelled arachidonic acid and a purified fraction of human plasma, the slopes of the concentration-response lines were all significant ( $P < 0.005$ , Table 2).

The time course of inhibition of PG synthesis by human serum was compared with that of aspirin, using two concentrations of each inhibitor (Fig. 1). Human serum exerted its maximal effect between 1 and 5 min and aspirin did so between 10 and 15 min after the start of incubation.

To eliminate the possibility that these inhibitory effects might largely be due to increased prostaglandin breakdown by the test serum, we studied the effect of the most potent serum tested (rat) on the main product of PG synthesis by bovine seminal vesicle homogenate (PGE<sub>2</sub>) in our experimental conditions. In two experiments, 0.25  $\mu$ Ci of <sup>3</sup>H-PGE<sub>2</sub> (specific activity, 140 Ci per mmol) was incubated with 0.5, 5.0 or 15.0% v/v rat serum for 15 min. The reaction was stopped with citric acid, prostaglandins were extracted with ethylacetate and separated by TLC in a modified AI system<sup>2</sup>. Areas corresponding to reference prostaglandins were scraped into scintillation vials and radioactivity in disintegrations per min estimated by liquid scintillation spectrometry. In the two experiments, percentage losses of tritiated PGE<sub>2</sub> were respectively:

0.5% serum, 0 and 30; 5% serum, 6 and 30; 15% serum, 0 and 26. In further experiments using the above conditions, the effect of various concentrations of a purified EIPS fraction of human plasma (purified Cohn IV-4, Table 2) instead of rat serum, on <sup>3</sup>H-PGE<sub>2</sub> and <sup>3</sup>H-PGF<sub>2 $\alpha$</sub>  (specific activity 15 Ci mmol<sup>-1</sup>) was investigated. In two experiments, percentage losses of tritiated PGE<sub>2</sub> were respectively: 50  $\mu$ g ml<sup>-1</sup> EIPS fraction, 0 and 2.4; 200  $\mu$ g ml<sup>-1</sup> EIPS fraction, 13.1 and 8.4; 500  $\mu$ g ml<sup>-1</sup> EIPS fraction 0 and 7.0. In similar experimental conditions, no loss of added <sup>3</sup>H-PGF<sub>2 $\alpha$</sub>  occurred. On the basis of these results we therefore concluded that the decrease in net production of prostaglandins in the presence of rat serum and purified EIPS fraction could mainly be attributed to the inhibition of PG synthesis rather than to activation of PG degradation.

### Activity of human plasma fractions

The ability of partially purified Cohn fractions I to VI of human plasma to inhibit PG synthesis by BSV was tested. Fractions I, II, III, V and VI were inactive at 1,500  $\mu$ g ml<sup>-1</sup> or lower concentrations; but fraction IV ( $\alpha$ -globulins) was active at 50, 500 and 1,500  $\mu$ g ml<sup>-1</sup>. Subfractions 1 and 4 of fraction IV, which were available, were then tested at 5–1,500  $\mu$ g ml<sup>-1</sup> (Table 2). Subfraction 4 was considerably more active than subfraction 1 and was, therefore, further purified (Table 2). This purified material and various specimens of Cohn IV-4 were therefore used in the pharmacological tests described below.

### Inhibition of adjuvant arthritis

As known inhibitors of PG synthetase ameliorate adjuvant arthritis in the rat<sup>4,5</sup>, we tested Cohn subfraction IV-4 against the swelling of the hind feet of rats that had received an intraplantar injection of Freund's adjuvant. Treatment with Cohn IV-4 lessened the extent of both the intense swelling of the

Table 2 Inhibitory activity of human plasma Cohn fractions IV, IV-1, IV-4 and purified IV-4 against PG synthesis

Concentration ( $\mu$ g ml <sup>-1</sup> )	Inhibition by (%)			Purified IV-4			
	IV	IV-1	IV-4	PGE <sub>2</sub>	PGF <sub>2<math>\alpha</math></sub>	PGE <sub>2</sub> *	PGF <sub>2<math>\alpha</math></sub> *
	Total PG production						
50	13.4±4.6	7.8±7.5	25.0±13.0	14.7±0.82	14.3±6.4	22.4±1.0	25.2±0.91
200	NT	NT	NT	42.1±18.8	69.9±18.5	35.2±2.10	39.5±0.95
500	23.8±2.6	10.5±5.8	53.0±7.7	94.9±3.4	97.4±2.6	88.5±5.90	83.1±3.10
1,500	77.3±3.2	39.4±0.96	73.0±1.8	NT	NT	NT	NT

Inhibition values are mean  $\pm$  s.e.m. Human plasma Cohn fractions IV, IV-1 and IV-4 were from Miles Laboratories. Purification of Cohn IV-4 was carried out by precipitation with 50% saturated ammonium sulphate, followed by chromatography on DEAE-cellulose, using a linear gradient of sodium acetate buffer (pH 5.0) from 0.01 to 0.5 M. Experiments with Cohn IV, IV-1 and IV-4 were performed using bioassay of total PG-like substances produced by incubating arachidonic acid with bovine seminal vesicle homogenate, as in Table 1. Experiments with purified Cohn IV-4 were performed using <sup>3</sup>H-arachidonic acid (specific activity 80 Ci mmol<sup>-1</sup>) and bovine seminal vesicle homogenate. The incubation mixtures, consisting of 1  $\mu$ Ci <sup>3</sup>H-sodium arachidonate (0.061 mM), reduced glutathione or noradrenaline, 0.5 ml bovine seminal vesicle homogenate and purified Cohn IV-4 as indicated, in 2 ml of 50 mM phosphate buffer (pH 7.4) were incubated in glass tubes at 37 °C for 15 min with gentle shaking. The reaction was stopped by adding 2 ml 0.2 M citric acid and 16 ml ethylacetate, and 100  $\mu$ g of unlabelled PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  were added as carriers. The radioactivity was quantitatively extracted in the ethylacetate phase, the extracts were evaporated to dryness, taken up in 400  $\mu$ l absolute ethanol and submitted to TLC on silica gel plates in the AI solvent system, with reference PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  as markers. After chromatography, the zones corresponding to PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  were scraped into scintillation vials and eluted with 1 ml ethanol. The radioactivity was determined by scintillation counting in Unisolve 1 (also containing 5 g l<sup>-1</sup> Cab-o-sil). To determine percentage inhibition of PG synthesis, radioactivity in c.p.m. of PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  in zones from test incubates was compared with that of controls.

\*Noradrenaline 1.3 mM used as cofactor instead of 1.3 mM reduced glutathione, which was used in all other incubates.

injected hind foot and the more gradual and less intense swelling of the uninjected foot (Fig. 2). Analysis of results (Mann-Whitney *U* test) on day 13 shows that, in comparison with saline, subfraction IV-4 both at 2 and 10 mg per kg body weight subcutaneously, given 1 d before adjuvant and then daily (except day 4) until day 11, significantly ( $P < 0.05$ ) reduced the swelling of the adjuvant-treated left hind foot and of the untreated right hind foot. The higher dose (10 mg per kg) also significantly ( $P < 0.05$ ) reduced the incidence of swelling of the fore feet and totally suppressed the incidence of nodules in the tail ( $P < 0.05$ ).

### Inhibition of bronchoconstriction

In 13 anaesthetised guinea pigs, purified Cohn subfraction IV-4, given intravenously (i.v.) in doses of 5, 20, 50 or 100 mg per kg, inhibited the increase of airway resistance and the decrease of lung compliance induced by i.v. injection of 0.1–0.5 mg per kg of arachidonate (Table 3). At doses effective in inhibiting bronchoconstriction induced by arachidonate, Cohn subfraction IV-4 did not inhibit bronchoconstriction induced by submaximal doses of  $\text{PGF}_{2\alpha}$  or acetylcholine (three guinea pigs).

Like aspirin<sup>6</sup>, indomethacin 33 and 100  $\mu\text{g}$  per kg i.v. inhibited the bronchoconstriction induced by i.v. arachidonate (Table 3), without inhibiting the response to  $\text{PGF}_{2\alpha}$  or acetylcholine.

For two reasons, we also tested the purified fraction of Cohn IV-4 against bradykinin-induced bronchoconstriction in the guinea pig. First, this bronchoconstriction is known to be mainly due to stimulation by bradykinin of prostaglandin production in the lungs<sup>7</sup>. Second, virtually the only drugs that suppress this effect are those that inhibit prostaglandin biosynthesis<sup>8</sup>.

Purified subfraction IV-4 inhibited the effect of intravenous challenge with bradykinin in this preparation (Table 3). In these tests indomethacin was considerably more potent than aspirin, whereas in previous experiments of this type, indomethacin had had unexpectedly low potency compared with aspirin<sup>6</sup>.

In preliminary experiments on human isolated bronchial muscle, prepared as previously described<sup>8</sup>, Cohn subfraction

IV-4, 0.1 and 1.0  $\text{mg ml}^{-1}$ , relaxed the muscle and inhibited the contraction induced by sodium arachidonate, 50  $\mu\text{g ml}^{-1}$ .

### Effect of corticosteroids on EIPS levels

In a preliminary experiment, 56 rats were given intraperitoneal (i.p.) dexamethasone sodium phosphate (0.1, 0.8 or 6 mg per kg); 4, 24, 48 and 72 h later rats were killed in groups of four or five, and the pooled serum of each group was tested for inhibitory activity. Dexamethasone raised, in a dose-related way, the ability of the serum to inhibit PG synthesis. This effect was apparent at 24 h after treatment, it reached a peak at 48 h and was considerably diminished at 72 h. In a similar experiment, using 72 rats, an i.p. dose of hydrocortisone sodium succinate (11, 90 or 360 mg per kg) likewise raised the inhibitory activity of the serum, with a comparable time course. In a third experiment, on 20 rats, aldosterone (0.2 mg per kg) failed to increase the inhibitory activity of the serum over a 72-h period.

In a further experiment on rats, dexamethasone sodium phosphate (24 mg per kg) or hydrocortisone sodium succinate (360 mg per kg) were given i.p. and 4, 24, 48 and 72 h later, animals were killed in groups of five and each bled individually from the dorsal aorta into tubes containing 0.2  $\text{mg ml}^{-1}$  of disodium EDTA as an anticoagulant. The plasma was separated by centrifugation for 15 min at 600*g* and the potency in inhibiting PG synthetase (EIPS level) of each plasma sample determined by the standard test procedure using bovine seminal vesicle homogenate (details as in Table 1). The results obtained showed that both dexamethasone and hydrocortisone raised plasma EIPS levels with comparable time courses (Fig. 3).

### Endogenous inhibitor of PG synthetase

We have presented evidence that incubation of mammalian blood plasma or serum with bovine seminal vesicle homogenate results in a lowered production of prostaglandins. There are several possible ways in which this could occur, but most of these are incompatible with the evidence. The effect cannot be due to inhibition of phospholipase A because (1) EDTA, which inhibits phospholipase-A, is present in the assay medium and (2), an optimal amount of sodium arachidonate was added to the medium. Neither can the effect be due to inhibition of

**Table 3** Inhibition of increased airway resistance and decreased lung compliance induced by sodium arachidonate or bradykinin

Treatment	Dose (mg per kg)	Challenge substance	% Inhibition of response to challenge*	
			Resistance	Compliance
Purified Cohn IV-4	5	Arachidonate sodium	31.8±4.7	24.4±5.3
	20		44.3±6.7	34.3±7.4
	50		67.7±0.3	49.0±3.2
	100		67.0±8.0	64.0±0
	5	Bradykinin	34.7±1.7	19.5±5.5
	20		48.3±4.4	33.4±11.8
	50		67.0±0	73.2±19.8
	100		94.0±6.0	86.5±13.5
Aspirin sodium	2	Arachidonate sodium	85.5±3.5	71.5±6.5
	2	Bradykinin	82.0±9.6	80.7±9.9
Indomethacin sodium	0.03	Arachidonate sodium	47.0±15.8	58.7±15.7
	0.1	Bradykinin	90.5±9.5	85.5±14.5
	0.01		35.5±9.9	25.3±8.2
	0.03		58.0±14.0	53.0±3.0
	0.1		88.5±11.5	86.0±6.0

Male albino guinea pigs (400–600 g, Dunkin Hartley strain) were anaesthetised with pentobarbitone sodium, 60 mg per kg i.p. The trachea was cannulated for artificial ventilation by a Starling pump (5–8 ml at 72 strokes per min) by a Fleisch 0000 pneumotachograph. The difference in pressure across this device was measured with a Grass differential pressure transducer. This gives a flow signal that was fed into a Hewlett-Packard 8816A analogue computer. Transpulmonary pressure (TPP) was measured with another differential pressure transducer via side-arms to the thoracic cavity and the tracheal cannula. The signals for flow and TPP were applied to the analogue computer so that there was approximately 180° phase difference. Dynamic compliance and airway resistance were derived by the computer from the ingoing signals. Drugs were administered intravenously through the external jugular vein. Arachidonic acid, 99% pure from Sigma was given in doses of 0.1–0.5 mg per kg as the sodium salt. Bradykinin as triacetate from Sigma was given at 1–5  $\mu\text{g}$  per kg. Test drugs were injected 45 s before challenge. Other details as in Table 2.

\*Values are mean ± s.e.m.

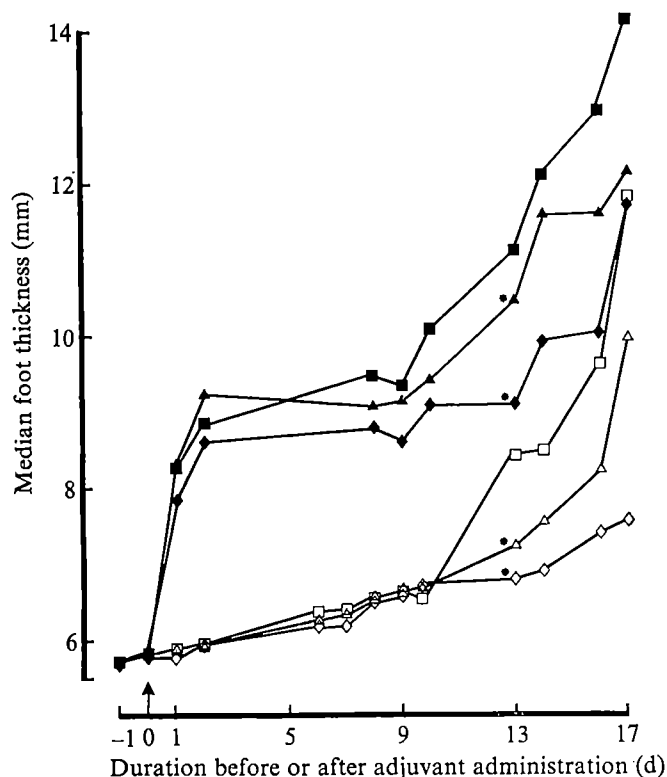


Fig. 2 Inhibition by Cohn subfraction IV-4 of adjuvant arthritis in the rat, induced by the method of Newbould<sup>4</sup>. A suspension of 25  $\mu$ g of killed, ground *Mycobacterium butyricum* (Difco) in 50  $\mu$ l of light liquid paraffin was injected into the plantar surface of the left hind foot of each of three groups of 12 rats on day 0 (arrow). On days -1 to 3 and days 5 to 11, saline (■) or subfraction IV-4, 2 mg per kg (▲) or 10 mg per kg (◆) was injected subcutaneously. Foot swelling was measured daily by micrometer, placed sagittally. Closed symbols, injected foot; open symbols, uninjected foot. \*,  $P < 0.05$  for difference of test from saline-treated controls (Mann-Whitney  $U$  test).

PG release from intact cells, because the cells of bovine seminal vesicle homogenate have already been disrupted. The experiments with tritiated  $PGE_2$  and  $PGF_{2\alpha}$  eliminate the possibility that the effect of serum and a purified fraction of plasma rich in EIPS activity was due to activation of enzymes destroying prostaglandins. We conclude that mammalian blood plasma and serum contain a mechanism for inhibiting PG synthetase. The presence of this mechanism in plasma indicates that it is not simply an artefact generated in serum during blood clotting. Since PG synthesis can be inhibited by antioxidants<sup>9,10</sup>, one of which (propyl gallate) has anti-inflammatory activity *in vivo*<sup>10</sup> and since macromolecules and macromolecular complexes have an undoubted, though little-understood, capacity to act as free radical stabilisers<sup>11,12</sup>, we have considered the possibility that the inhibitory effect might be a nonspecific property of blood proteins. This possibility was eliminated by the finding that this effect of plasma was restricted to the  $\alpha$ -globulin fraction, representing about 5% of total plasma proteins. We therefore postulate that plasma and serum contain an endogenous factor (or factors) that potently inhibits PG synthetase (EIPS). Analysis of its biochemical properties showed EIPS to be a protein, because neither deproteinised plasma nor serum inhibited PG synthesis and because EIPS was inactivated by Pronase. EIPS could not be dialysed through a Cellophane membrane, indicating a large molecular size. Further characterisation of human plasma EIPS has shown that its activity is associated with haptoglobins (in preparation). Smith *et al.*<sup>13</sup> have previously described an anti-inflammatory fraction of human plasma. This differs in two main ways from EIPS, however. First, this fraction does not inhibit prostaglandin synthesis; second, it is of relatively low molecular weight (about 1,000).

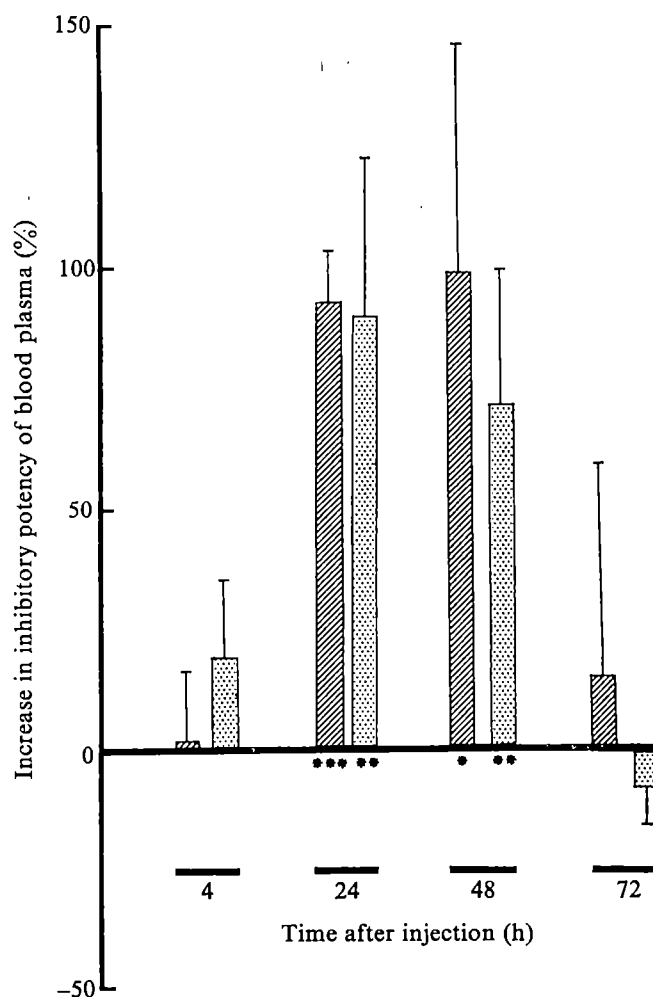
These observations raise the question: does EIPS represent a natural mechanism for control of PG synthesis? If so, how is EIPS inactivated when a need for PG synthesis arises? If so, again, what is the relevance of EIPS to diseases associated with excessive or unbalanced PG production?

### Anti-inflammatory drugs

By analogy with morphine, which may be regarded as a mimic of an endogenous ligand of the opiate receptor<sup>14,15</sup>, the possibility arises that non-steroidal anti-inflammatory drugs, which inhibit PG synthetase, may act as mimics of EIPS. Other relationships between these drugs and EIPS are possible, however.

Several studies have shown that corticosteroids interfere with the release or production of prostaglandins or related substances in preparations that include human skin homogenate<sup>16</sup>, dog hind leg<sup>17</sup>, adipose tissue<sup>18</sup>, mesenteric artery<sup>19</sup>, cultured synovial cells<sup>20,21</sup>, cultured fibrosarcoma cells<sup>22</sup>, and isolated guinea-pig lungs in anaphylaxis<sup>23</sup>. In most of these studies the mechanism by which corticosteroids acted was not fully identified; but Nijkamp *et al.*<sup>23</sup> showed that corticosteroids, with a relative potency comparable to their anti-inflammatory activity *in vivo*, interfere with the release of arachidonate, induced by rabbit aorta contracting substance-releasing factor (RCS-RF).

Fig. 3 Changes in EIPS levels of blood plasma taken from rats at various times after the intraperitoneal injection of dexamethasone or hydrocortisone. Cross-hatched column, 360 mg per kg hydrocortisone sodium succinate; stippled column, 24 mg per kg dexamethasone sodium phosphate. Each column represents the mean  $\pm$  s.e.m. of five determinations. For other details see text and Table 1. \*,  $P < 0.05$ ; \*\*,  $P < 0.025$ ; \*\*\*,  $P < 0.0005$  for difference from saline-treated controls (Student's  $t$  test).



In the experiments described here we have shown that, in normal rats, administration of a natural or a synthetic anti-inflammatory glucocorticoid (hydrocortisone or dexamethasone), but not of the mineralocorticoid, aldosterone, increased the ability of the plasma or serum to inhibit prostaglandin synthesis by bull seminal vesicle homogenate. This effect is presumably due to the increase of EIPS. This suggests that glucocorticoids may act, at least in part, by increasing EIPS.

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1. Collier, H. O. J. *Nature* 232, 17–19 (1971); in *Prostaglandin Synthetase Inhibitors* (eds. Robinson, H. J. & Vane, J. R.) 121–133 (Raven, New York, 1974).
2. Collier, H. O. J., McDonald-Gibson, W. J. & Saeed, S. A. *Br. J. Pharmac.* 58, 193–199 (1976).
3. Ubatuba, F. B. *Br. J. Pharmac.* 49, 662–666 (1973).
4. Newbould, B. B. *Br. J. Pharmac.* 21, 127–136 (1963).
5. Ward, J. R. & Cloud, R. S. *J. Pharmac. exp. Ther.* 152, 116–121 (1966).
6. Collier, H. O. J. *Adv. Pharmac. Chemother.* 7, 333–404 (1969); in *Proc. Fogarty Int. Symp. on Chemistry and Biology of the Kallikrein-Kinin System in Health and Disease* (ed. Pisano, J. J.) 495–503 (US Government Printing Office, Washington, D.C., 1976).

7. Vane, J. R. & Ferreira, S. H. in *Proc. Fogarty Int. Symp. on Chemistry and Biology of the Kallikrein-Kinin System in Health and Disease* (ed. Pisano, J. J.) 255–266 (US Government Printing Office, Washington, D.C., 1976).
8. Gardiner, P. J. *Prostaglandins* 10, 607–616 (1975).
9. Lands, W. E. M., Letellier, P. R., Rome, L. H. & Vanderhoeck, J. Y. *Adv. Biosci.* 9, 15–28 (1973).
10. McDonald-Gibson, W. J., Saeed, S. A. & Schneider, C. *Br. J. Pharmac.* 58, 573–581, (1976).
11. Matushita, S., Ileuki, F. & Aoki, A. *Archs. Biochem. Biophys.* 102, 446–451 (1963).
12. Dormandy, T. L. *Br. J. Haemat.* 20, 257–261 (1971).
13. Smith, M. J. H., Ford-Hutchinson, A. W., Elliott, P. C. N. & Bolam, J. P. *J. Pharm. Pharmac.* 26, 692–698 (1974).
14. Collier, H. O. J. in *Proc. 5th Int. Congr. Pharmac. San Francisco 1972* 1 (eds. Cochlin, J. & Leong Way, E.) 65–76 (Karger, Basle, 1973).
15. Hughes, J. *et al. Nature* 258, 577–579 (1975).
16. Greaves, M. W. & McDonald-Gibson, W. J. *Br. J. Pharmac.* 46, 172–175 (1972).
17. Herbaczynska-Cedro, K. & Staszewska-Barczak, J. *Abs. 2nd Congress of the Hungarian Pharmacological Society*, 19 (Budapest, 1974); *Prostaglandins* 13, 517–531 (1977).
18. Lewis, G. P. & Piper, P. J. *Nature* 254, 308–311 (1975).
19. Gryglewski, R. J., Panzenko, B., Korbut, R., Grodzinska, L. & Ocetkiewicz, A. *Prostaglandins* 10, 343–355 (1975).
20. Kantrowitz, F., Robinson, D. W., McGuire, M. B. & Levine, L. *Nature* 258, 737–739 (1975).
21. Floman, Y. & Zor, U. *Prostaglandins* 12, 403–413 (1976).
22. Tashjian, A. H., Voelkel, E. F., McDonough, J. & Levine, L. *Nature* 258, 739–741 (1975).
23. Nijkamp, F. P., Flower, R. J., Moncada, S. & Vane, J. R. *Nature* 263, 479–482 (1976).

# letters to nature

## A search for the reported 400-keV $\gamma$ -ray line from Crab Nebula

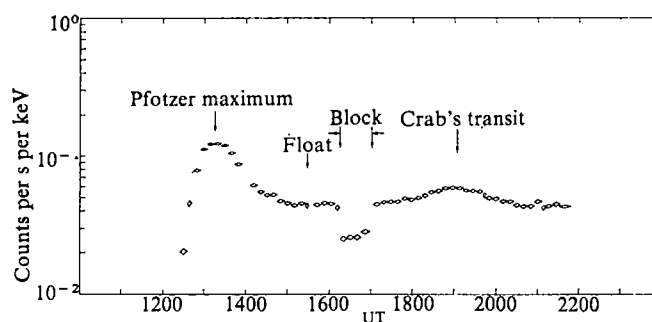
LEVENTHAL *et al.*<sup>1</sup> have reported a possible  $\gamma$ -ray line at  $400 \pm 1$  keV from the Crab Nebula. The measurement was achieved with a balloon-borne high resolution  $\gamma$ -ray telescope flown from Alamogordo, New Mexico on 10–11 May 1976. The intensity and the width of the line are reported to be  $(2.24 \pm 0.65) \times 10^{-3}$  photon  $\text{cm}^{-2} \text{s}^{-1}$  and  $\lesssim 3$  KeV, respectively. These authors suggested that a possible origin of this line may be the gravitational redshift of the 0.511 MeV annihilation line produced near the surface of the neutron star. The line, if real, is of great astrophysical interest, and a confirmation of the measurement is therefore important. We report here the results of our search for this feature.

On 10 June 1974 we conducted a balloon experiment over Palestine, Texas with a large volume high resolution  $\gamma$ -ray spectrometer. The instrument which has been described previously<sup>2</sup> consisted of four 40-cm<sup>3</sup> Ge(Li) crystals (only three were operational for the flight) operating in the energy range of 0.06–10 MeV. This cluster of detectors is surrounded by a CsI(Na) anticoincidence shield for reducing the background and providing collimation of 25° FWHM at 400 keV. The total effective area (efficiency  $\times$  front area) is 10.4 cm<sup>2</sup> and the energy resolution is 2.2 keV FWHM at the same energy. The objectives of the experiment were to measure (1) X-ray and  $\gamma$ -ray emissions from the Crab Nebula, (2) diffuse cosmic fluxes (3) atmospheric  $\gamma$  rays and (4) the instrument's background in a space environment. A study of the 0.511 MeV  $\gamma$  rays from the terrestrial and extraterrestrial environments, results of the observation of a 'flare-like'  $\gamma$ -ray line event, and the hard X-ray spectrum (53–300 keV) of the Crab Nebula are reported elsewhere<sup>3–5</sup>. A detailed analysis of the  $\gamma$ -ray spectrum (0.3–10 MeV) of the Crab Nebula apart from the 400 keV and 511 keV lines is now underway, and will be reported in the future.

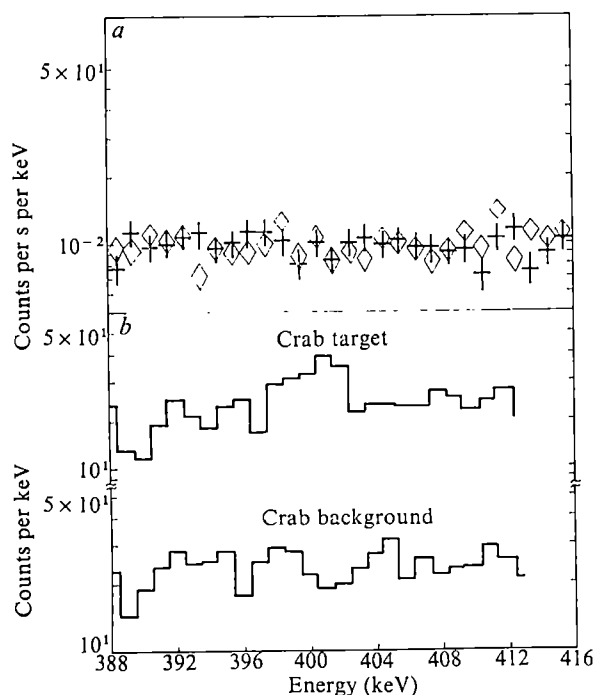
The balloon was launched at 1204 UT and reached a float altitude of 2.9 g cm<sup>-2</sup> at approximately 1530 UT where it remained for 6.5 h before shutdown. During the entire flight, the azimuth servo system maintained the instrument's

aperture in a southerly direction and the elevation was set 10° from the zenith, allowing the Crab Nebula and the Sun, separated by 4°, to transit directly through the field of view. Since the viewing axis azimuth and zenith angles were fixed, background variations which may be associated with changes in these parameters by pointing the instrument towards and away from the target such as the method used by Leventhal *et al.*<sup>1</sup> are therefore eliminated. Background variations owing to the balloon spatial drifts are estimated to be  $\sim 1\%$ . Figure 1 shows the time history of the counting rate measured by the three Ge(Li) detectors in the range 65–100 keV. During the first part of float, the aperture was blocked with a 20.3 cm diameter  $\times$  10.2 cm thick NaI(Tl) crystal also in anticoincidence with the prime sensors and the rate decreased from 1.6 counts per s to 0.9 counts per s. The difference can be attributed to  $\gamma$  rays originating from the atmospheric and diffuse cosmic components and entering through the aperture. The Sun and the Crab Nebula transited through the field of view at 1845 UT and 1902 UT, respectively. By 1902 UT the rate had increased to 27% over

Fig. 1 Time history of the counting rate measured by three of the four Ge(Li) detectors (65–100 keV). During the first part of float at 2.9 g cm<sup>-2</sup>, the aperture was covered by a 20.3 cm diameter  $\times$  10.2 cm thick NaI(Tl) detector and the rate decreased from 1.6 to 0.9 counts per s. The difference can be attributed to aperture fluxes which consist of the atmospheric and diffuse cosmic X-rays. The Crab Nebula transited directly over the field of view at 1902 UT.







**Fig. 2** Energy loss spectra. Our measured rates around 400 keV (a) are compared with those reported by Leventhal *et al.*<sup>1</sup> during live-time intervals of 7,113-s (Crab target) and 7,084-s (Crab background), respectively (C. J. MacCallum, personal communication). Their measured line flux of  $2.24 \times 10^{-3}$  photons  $\text{cm}^{-2} \text{s}^{-1}$  should increase our measured rate in the 398–403 keV bin of the Crab plus Background spectrum by  $1.26 \times 10^{-2}$  counts per s. Such an increase of  $3.9\sigma$  statistical significance is not observed in our data. In a +, Crab plus background, 104 min;  $\diamond$ , background, 128 min.

the background measured before and after the transits. Since the Solar Geological data (No. 360–Part 1, 1974) indicated no significant X-ray and  $\gamma$ -ray activity during this period, contributions to the enhancement of the measured rate from the Sun are expected to be negligible.

Figure 2a shows two energy-loss spectra in the range 388–412 keV with one of the spectra accumulated over a 104 min (1812–1956 UT) live-time interval during the Crab Nebula transit and the other over a 128 min live-time interval before (1702–1742 UT) and after (2016–2148 UT) the transit. The length of the source transit-interval selected for analysis was chosen such that the ratio of the total number of counts from the Crab Nebula over its statistical significance was maximised. The net average exposed effective area of the instrument to the Crab Nebula during the transit was  $5.62 \text{ cm}^2$  at 400 keV. This value is determined experimentally using laboratory radioactive sources and computation with the Monte Carlo technique<sup>3,6</sup> and further corrected for effects of attenuation of the overlying atmosphere and materials in the gondola, balloon spatial drift<sup>3</sup> and leakages through the shield. Also shown in Fig. 2 for comparison are the corresponding spectra measured by Leventhal *et al.*<sup>1</sup> (C. J. MacCallum, personal communication). During the Crab Nebula transit the difference in our data of the counting rate measured in the 398–403 keV bin and the average rate measured in the two adjacent bins 388–398 keV and 403–413 keV is  $(-2.85 \pm 3.27) \times 10^{-3}$  counts per s. The null result is different from the measurement of Leventhal *et al.* of a positive increase of  $4.0\sigma$  significance using the same bin selection. Their reported line flux of  $2.24 \times 10^{-3}$  photons per  $\text{cm}^2$  per s from the Crab Nebula would increase our measured rate in the 398–403 keV bin of the Crab plus background spectrum by  $1.26 \times 10^{-2}$  counts per s. Such an increase of  $3.9\sigma$  statistical significance is clearly not observed in our data. In addition, our background spectrum with the Crab Nebula outside the field

of view shows rates in the same three energy bins similar to those accumulated during the source transit. As these results are also consistent with our prediction of background variation of 1% owing to balloon spatial drifts, we may rule out the possible explanation that our null results in observing the 400 keV line is caused by systematic background effects.

In conclusion, we did not observe the 400-keV  $\gamma$ -ray line from the Crab Nebula on 10 June 1974. Our result is in contradiction with Leventhal's measurement if a constant source intensity is assumed. The two observations were separated by 2 yr, however, and we cannot rule out the possibility that the feature may vary with time. Observations with a high resolution instrument may resolve this issue.

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1. Leventhal, M., MacCallum, C. J. & Watts, A. C. *Nature* **266**, 696 (1977).
2. Jacobson, A. S. *et al. Nuclear Instr. Meth.* **127**, 115 (1975).
3. Ling, J. C., Mahoney, W. A., Willett, J. B. & Jacobson, A. S. *J. geophys. Res.* **82**, 1463–1473 (1977).
4. Jacobson, A. S., Willett, J. B., Ling, J. C. & Mahoney, W. A. *Astrophys. J. Lett.* (to be submitted).
5. Jacobson, A. S., Ling, J. C., Mahoney, W. A. & Willett, J. W. *Astrophys. J. Lett.* (to be submitted).
6. Ling, J. C. thesis, Univ. California, San Diego (1974).

## Hadronic photoabsorption and pair production in pulsars

THERE are two existing models for the acceleration of positive charged particles near the magnetic polar caps of a pulsar. In the first, the work function for a  $^{56}\text{Fe}$  ion bound in the lattice at the stellar surface is assumed, following exhaustive calculations of Flowers *et al.*<sup>1</sup>, to be too large for the occurrence of ion emission. Electron–positron pairs are created through magnetic conversion of curvature photons<sup>2</sup>; the electrons are accelerated inwards to the stellar surface, and the positrons outwards along open lines of magnetic flux to the light cylinder. In the second, the work function is assumed to be negligible and ion emission limited only by space charge effects. In the corotating frame of reference, all components of the surface electric field are zero and inertial ion acceleration<sup>3–5</sup> occurs. For surface magnetic flux densities  $B \gtrsim 10^{12} \text{ G}$ , we shall attempt to show here that the nature and mode of acceleration of the plasma moving outwards along open magnetic flux lines are determined, not by these processes, but by the occurrence of hadronic photoabsorption reactions in the electromagnetic showers produced by ultra-relativistic electrons incident on the stellar surface. This work was prompted by the comment<sup>4</sup> that magnetic conversion of backward moving photons from these showers may be an important source of pairs. The consequences of hadronic photoabsorption do not seem to have been considered previously. Here, it is shown that photons of the energy necessary for conversion in a magnetic flux density of  $10^{12} \text{ G}$  are emitted with high probability in hadronic photoabsorption reactions, and an equation is obtained for the acceleration potential difference in a one-dimensional model of electron–positron pair creation at the magnetic polar cap. The properties of the accelerated plasma are important owing to its probable

connection with radio emission and with the phenomenon of subpulse drift occurring in certain pulsars (see, for example, refs 6, 7).

Consider a photon emitted, for example, at an angle of  $150^\circ$  to the direction of the primary electron, assumed parallel with  $\mathbf{B}$ . For  $B = 10^{12}$  G, the mean free path for magnetic conversion<sup>8</sup> is in the interval  $1 \leq \lambda \leq 10^4$  cm for photon energy  $\epsilon$  such that  $9 \geq \epsilon \geq 5$  MeV. A photon of these properties would have a negligible probability of creation by purely electromagnetic processes, as shown by the shower distribution function tables of Messel and Crawford<sup>9</sup>.

The transverse momentum of most photons in the shower is  $\lesssim 10 mc$ , defined with respect to  $\mathbf{B}$ , and therefore the properties of the shower should be unchanged by the magnetic flux. From the shower distributions<sup>9</sup> (for air) the photon track length at energies of more than 300 MeV is estimated to be  $1.6 W$  radiation lengths, where  $W$  is the primary electron energy in GeV. For  $W \approx 10^3$  GeV, several photoabsorption reactions would occur per shower. The equilibrium state of matter at the stellar surface would be determined by the formation of light nuclei (with negligible work functions) in the spallation of  $^{56}\text{Fe}$  and their emission from the surface by thermal excitation. At the temperatures and densities considered, fusion and diffusion would seem unimportant. Owing to the spallation and the interaction-induced disorder, the matter would have the characteristics of a glassy solid. However large the  $^{56}\text{Fe}$  work function, the plasma moving outwards must have an ionic component. The charge spectrum of light nuclei accelerated would include  $Z = 3-5$ , normally considered to be present in galactic cosmic rays as a result of the interaction of heavier nuclei with the interstellar medium.

For photon energies below the meson threshold, the most important photoabsorption reaction is the formation of the giant dipole resonance. The cross section, integrated over photon energy from 15 to 30 MeV, is a large fraction of the classical sum rule result<sup>10</sup>,  $60(NZ/A)$  mb MeV. For mass numbers  $A > 40$ , neutron emission is the dominant decay mode of the resonance. Proton emission occurs, but is less probable. Photons with energy in the interval  $5 < \epsilon < 9$  MeV are produced with approximately unit probability in both the further decay of the residual nucleus and the capture of the neutron. The average number of photons from these processes entering the magnetosphere with energy such that their mean free path for magnetic conversion is in the interval  $1 < \lambda < 10^4$  cm can be estimated, from the shower distributions<sup>9</sup>, to be of the order of 1–10 for a primary electron of  $10^3$  GeV. A laboratory measurement of the number and energy spectrum should be possible at the lower energies of existing electron beams.

We later summarise some results for a one-dimensional model of pair creation by this mechanism. It is assumed that acceleration occurs for  $0 < z < z_a$ , where  $z$  is the distance from the stellar surface measured along an open line of magnetic flux at the polar cap, and  $z_a \lesssim 10^4$  cm. In the corotating frame of reference, the potential  $\phi(z)$  satisfies the condition

$$\phi(0) = \left( \frac{\partial \phi}{\partial z} \right)_{z_a} = 0 \quad (1)$$

In units of  $\sigma_0 c$ , where  $\sigma_0$  is the (positive) corotational charge density<sup>11</sup>, the ion current density is  $\alpha_1$ , and the electron-positron current density resulting from magnetic conversion between  $z_0$  and  $z_a$  is  $\alpha_2$ . The lower limit  $z_0 \ll z_a$  represents a maximum photon energy. It is assumed that  $\alpha_1 + \alpha_2 = \alpha \leq 1$ .

The first result obtained is that the mean number of photon conversions per primary electron and unit interval of  $z$  is of the form

$$G(z) \propto z^{-1}, \quad z_0 < z < z_a \quad (2)$$

as a function of  $z$ . This is independent of the photon angular distribution, is exact if the photon energy spectrum is  $f(\epsilon) \propto \epsilon^{-2}$ ,

and is a satisfactory approximation for any reasonable  $f(\epsilon)$ . It is assumed, on the basis of the discussion of the giant dipole resonance, that the form of  $f(\epsilon)$  (though not its scale) is independent of primary electron energy. With this result, the potential  $\phi(z)$  satisfying the boundary conditions (1) can be found from Poisson's equation, assuming all particle velocities equal to  $c$ . The maximum acceleration potential is

$$\phi(z_a) = -2\pi\sigma_0 z_a^2 (1 - \alpha + \alpha_2 / \ln(z_a/z_0)) \quad (3)$$

Following the discussion of hadronic photoabsorption, we assume that the mean number of photon conversions between  $z_0$  and  $z_a$  per electron created at  $z$  and accelerated inwards to the stellar surface is

$$F(z) = C\phi(z), \quad z_0 < z < z_a \\ = 0, \quad z < z_0 \quad (4)$$

where the lower limit  $z_0$  satisfies  $z_0 \ll z_0 \ll z_a$  and  $C$  is a function of this lower limit and of  $B$ . The equation for  $\phi(z_a)$  can be derived from the condition for a time-independent electron-positron current density. It is

$$4\phi(z_a)/\phi(z_0) = \exp(2F(z_a)), \text{ in the limit } \alpha \rightarrow 1 \quad (5)$$

or

$$2\phi(z_a)/\phi(z_0) = \exp(\frac{1}{2}F(z_a)), \quad 1 - \alpha \gg \alpha_2 / \ln(z_a/z_0) \quad (6)$$

The solutions of equations (5) and (6) are insensitive to  $z_0$  and as functions of  $C$  are approximately  $\phi(z_a) \propto C^{-1}$ . We estimate  $-\epsilon\phi(z_a) \approx 10^3$  GeV for  $B = 10^{12}$  G on the basis of the earlier (order of magnitude) discussion of photon production. Thus pair production and the outward flow of positrons may occur through this mechanism rather than the conversion of curvature radiation which would require a small radius of curvature ( $< 10^7$  cm) for the open magnetic flux lines at the stellar surface. Both a laboratory measurement of photon production and a detailed study of the effect of the magnetic flux on shower properties, however, are necessary for quantitative estimates of  $C$  and  $\phi(z_a)$ .

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1. Flowers, E. G. *et al. Astrophys. J.* **215**, 291 (1977).
2. Ruderman, M. A. & Sutherland, P. G. *Astrophys. J.* **196**, 51 (1975).
3. Michel, F. C. *Astrophys. J.* **192**, 713 (1974).
4. Cheng, A. F. & Ruderman, M. A. *Astrophys. J.* **214**, 598 (1977).
5. Fawley, W. M., Arons, J. & Scharlemann, E. T. *Astrophys. J.* **217**, 227 (1977).
6. Ritchings, R. T. & Lyne, A. G. *Nature* **257**, 293 (1975).
7. Taylor, J. H., Manchester, R. N. & Huguenin, G. R. *Astrophys. J.* **195**, 513 (1975).
8. Erber, T. *Rev. Mod. Phys.* **38**, 626 (1966).
9. Messel, H. & Crawford, D. F. *Electron-Photon Shower Distribution Function Tables* (Pergamon, Oxford, 1970).
10. Hayward, E. in *Nuclear Structure and Electromagnetic Interaction* (ed. N. MacDonald) (Oliver & Boyd, Edinburgh, 1965).
11. Goldreich, P. & Julian, W. H. *Astrophys. J.* **157**, 869 (1969).

## A classification scheme for pulsars

WE have already<sup>1</sup> distinguished between three types of pulsars depending upon their mode of formation. Here we discuss some implications of this classification scheme. We suggest the nomenclature types L, S and D for the three types of pulsars. Type S (S for single) consists of pulsars formed as a result of supernova explosions of single stars. Types D and L refer to pulsars which come from massive close binary systems. Pulsars produced by

supernova explosions in and leading to the disruption of the binaries belong to type D (D for disrupted), while the old, rejuvenated neutron stars liberated from binaries by the supernova explosions of the companion stars constitute type L (L for liberated). Type D consists of two subsets; a pulsar belongs to type D<sub>1</sub> if its formation, accompanied by the disruption of the binary, is a result of the supernova explosion of the first (originally the more massive) star; to type D<sub>2</sub>, if a result of the supernova explosion of the second (originally the less massive) star. Thus type D<sub>1</sub> pulsars are associated with OB runaways, while type D<sub>2</sub> pulsars are associated with type L pulsars.

This distinction between the three types of pulsars has two significant implications. First, the age of a type L pulsar is not simply determinable from its spin-down rate. Pulsars in massive close binaries are born in rapid rotation like single pulsars<sup>2</sup>, but slow down rapidly<sup>3</sup>. The neutron star spins up due to mass accretion from the companion star and mass loss from the system<sup>4</sup>. In terms of the concept of spin-down age, the neutron star ages rapidly immediately after its formation but is rejuvenated later. Only if the type L pulsar is spinning as rapidly at the time of the disruption of binary, as it was at the time of its formation, is its spin-down age relevant. For type D pulsars, of course, it is as good a measure of the true age as it is for type S pulsars.

Second, a type L or a type D<sub>2</sub> pulsar cannot be assumed to have always been moving with its present velocity, which is the orbital velocity the pulsar had at the time of the disruption of the binary by the second supernova explosion. Before that the system was moving with the velocity it had acquired as a result of the first supernova explosion. An estimate of the velocity can be made from the galactic *z*-distribution of massive X-ray binaries—a stage reached by the OB binary some 4–5 Myr after the first supernova explosion<sup>5</sup>, which can be assumed to have taken place in the galactic plane. As the average distance of massive X-ray binaries above the galactic plane is  $\sim 90$  pc (ref. 6), we conclude that the centre of mass of the system is accelerated to a *z*-velocity of  $\sim 20$ – $30$  km s<sup>−1</sup> by the first supernova explosion.

Since the time interval between the first and the second supernova explosion is about 5–7 Myr (ref. 5), the binary would have moved a distance 100–200 pc perpendicular to the galactic plane before being disrupted by the second supernova explosion. Thus while type S and type D<sub>1</sub> pulsars are born close to the galactic plane, type D<sub>2</sub> and L pulsars are born at large  $|z|$  values. If, however, the space velocity of the pulsar at the time of the disruption of the progenitor binary had a *z*-component, the present *z*-position of the pulsar could be above or below the *z*-value at which the pulsar was formed. Further, since the space velocities of type L and type D pulsars are typically an order of magnitude larger than the centre of mass velocities of their progenitor binaries, a pulsar could move a substantial distance in the *z*-direction after the disruption of the binary so that although it was formed at a large  $|z|$  value, it could now be close to the galactic plane. It could even have crossed the galactic plane and may now appear on the other side. Thus while a type L or a type D<sub>2</sub> pulsar is born at  $|z| \sim 100$ – $200$  pc, its present distance from the galactic plane would depend upon the magnitude and the sign of the *z*-component of its space velocity and on its age as a single object.

We can now review the galactic *z*-distribution of some pulsars. PSR 1133+16 has a transverse velocity of  $320$  km s<sup>−1</sup> at a height  $z = 150$  pc above the galactic plane<sup>7</sup> and a spin down age of 5 Myr. According to ref. 1 it is a type L pulsar. Two hypotheses can then explain its present *z*-position. (1) The progenitor binary had moved a *z*-distance of about 150 pc between the first and the second supernova explosions so that the pulsar was formed at a height  $\sim 150$  pc above the galactic plane and is now moving parallel to it. In that case its radial velocity should be  $\sim -120$  km s<sup>−1</sup>. (2) Or, the pulsar was born  $\sim 150$  pc below the galactic plane. The orbital plane of the progenitor binary was suitably inclined to the galactic plane to give the liberated pulsar an appropriate *z*-velocity. Some time ago, the pulsar must have crossed the galactic plane and is moving up. A similar explanation holds for PSR2021+51 which has a transverse velocity  $\lesssim 400$  km s<sup>−1</sup> but is only 120 pc above the galactic plane<sup>7</sup>. PSR 0823+26, PSR0834+06 and

PSR1237+25 are respectively 420, 210 and 370 pc above the galactic plane and have transverse velocities 506, 118 and 178 km s<sup>−1</sup> respectively<sup>8</sup>. They are all type L pulsars<sup>1</sup>. They must have been born  $\sim 150$  pc above the galactic plane and are moving up.

PSR1929+10 is located at  $z = -7$  pc and has a velocity  $75$  km s<sup>−1</sup> perpendicular to the galactic plane<sup>7</sup>. Since its velocity is much too small for its status as a type L pulsar<sup>1</sup> it must have a large radial velocity. The present location of the pulsar can be explained if we assume that it was born  $\sim 150$  pc below the galactic plane and is now on its way up. PSR0329+54 has a transverse velocity of  $\lesssim 450$  km s<sup>−1</sup> and is located at  $z = -28$  pc (ref. 7). We have classified it as a type D pulsar<sup>1</sup>. Either it is a type D<sub>1</sub> pulsar born at this *z*-value and is moving parallel to the galactic plane, or, alternatively, it is a type D<sub>2</sub> pulsar which was born at a large *z*-value and has since moved closer to the galactic plane. PSR0950+08 has a transverse velocity of  $50$  km s<sup>−1</sup> at  $z = 71$  pc (ref. 7). Since it is a type D pulsar<sup>1</sup>, it must have a large radial velocity. Further to explain its low *z*-value, in spite of its large age (17 Myr), it must be postulated that the pulsar was born at this *z*-value and is moving parallel to the galactic plane. The Vela pulsar 0833−45 is at a low value of  $z$  ( $-24$  pc) as a young type S pulsar should be. Another type S pulsar, PSR2016+28, is also close to the galactic plane ( $z = -33$  pc)<sup>8</sup>. But since it is very old (59 Myr) it must be moving parallel to the galactic plane. The Crab pulsar is located 200 pc below the galactic plane. Since it is an extremely young type S pulsar<sup>1</sup>, it has not moved much from its place of birth. Therefore its progenitor could not have been an OB star, all of which are confined to the galactic plane. Thus, Crab pulsar must be the result of the supernova explosion of a late type, low mass star.

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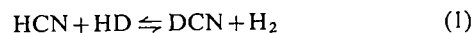
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Received 31 May; accepted 1 September 1977.

1. Kochhar, R. K. *Nature* **267**, 231 (1977).
2. Savonije, G. J. & Van den Heuvel, E. P. J. *Astrophys. J.* **214**, L19 (1977).
3. Kundt, W. *Phys. Lett.* **57A**, 195 (1976).
4. Kruszewski, A. *Adv. Astr. Astrophys.* **4**, 233 (1966).
5. Van den Heuvel, E. P. J. *Proc. 16th Solway Conf.* 119 (University of Brussels Press, Brussels, 1974).
6. Gursky, H. & Schreier, E. in *Neutron Stars, Black Holes and Binary X-ray Sources* (eds Gursky, H. & Ruffini, R.) (Reidel, Dordrecht, 1975).
7. Backer, D. C. & Sramek, R. A. *Astr. J.* **81**, 430 (1976).
8. Anderson, B., Lyne, A. G. & Peckham, R. J. *Nature* **258**, 215 (1975).

## Deuterium enrichment in interstellar HCN and HNC

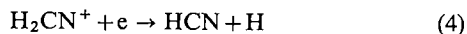
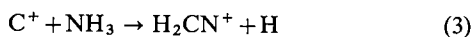
THE cosmic abundance of deuterium, expressed as the ratio D/H, is thought to be approximately  $2 \times 10^{-5}$  (ref. 1). Observations on deuterium-containing molecules, such as DCN (refs 2,3) and DNC (ref. 4), however, indicate much higher values for the molecular ratios DCN/HCN and DNC/HNC. In the case of DCN this was attributed to chemical enrichment involving equilibria such as<sup>5</sup>



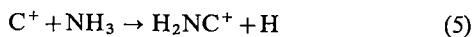
or to other mechanisms<sup>6</sup>. But it is difficult to reconcile the observed enrichment of DNC with any of these suggestions. Here we explore the alternatives and correct previous suggestions about the mechanism of formation of interstellar HCN. We consider conditions appropriate to dense molecular clouds such as that in Ori A or NGC2264 where the visual/ultraviolet optical depth is very high and ionisation is predominantly by cosmic rays.

For such clouds ion-molecule reactions in the gas phase<sup>7,8</sup> seem to account satisfactorily for many of the observations on molecular species and we shall base our discussion on this theme. Reactions on dust grains, especially very small ones, may also

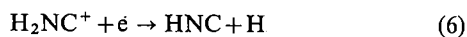
play a significant part<sup>9</sup>. In it, the formation of HCN is attributed to the following reactions:



In the low temperature conditions that prevail in interstellar molecular clouds, however, the N–H bonds will not be disrupted in equation (3) and so it should be written

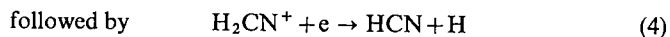
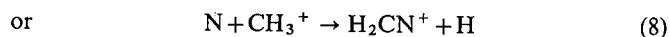
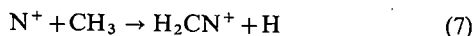


which would be followed by

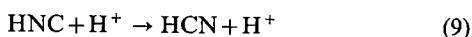


Thus the ion–molecule reaction between  $\text{C}^+$  and ammonia leads to HNC rather than HCN. It is often stated that  $\text{H}_2\text{CN}^+$  has the structure  $\text{HCNH}^+$ . While this seems to be the most stable isomer<sup>10</sup> there is no reason to doubt that the other isomers  $\text{H}_2\text{CN}^+$  and  $\text{H}_2\text{NC}^+$  correspond to alternative, locally stable arrangements. Unimolecular rearrangements of these less stable isomers to  $\text{HCNH}^+$  would be exceedingly slow at the temperatures prevailing in molecular clouds.

In place of equations (3) and (4) as the main means of producing HCN, the following scheme seems to be the most plausible



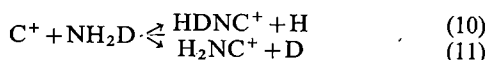
In addition to these processes there is the following rapid isomerisation channel for HNC which, of course, is another process for production of HCN:



Calculations on the relative stability of HCN and HNC (ref. 11) imply that the former is more stable by 0.63 eV and this is supported by some laboratory studies of the equilibrium (ref. 12 and unpublished results) so that the equilibrium ratio HNC/HCN is about  $10^{-30}$  at 100 K. The fact that column densities of HNC comparable to those of HCN have been observed in dark clouds indicates that equilibrium between the two species is not established. This is an important observation because its consequence is that related equilibria involving HNC and HCN in proton exchanges likewise will not be established (they would have to be established at rates much faster than equation (9) which is expected to occur at the Langevin rate and with one of the most abundant ionic species in dark clouds). Also because the equilibrium involving equation (9) has as fast a rate constant and as high concentrations (according to the numerical results of the ion–molecule reaction scheme obtained by Herbst and Klemperer, ref. 7) as any other process considered in discussions of deuterium enrichment through chemical equilibration, it is difficult to sustain such interpretations of molecular deuterium enrichment in dense molecular clouds.

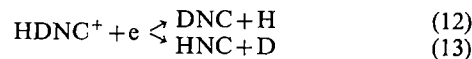
We conclude that the explanation of molecular deuterium enrichments in interstellar HCN and HNC must lie in isotope effects that influence the formation processes rather than equilibria involving HCN or HNC.

In the case of HNC the deuterium is introduced, according to reactions (5) and (6) through  $\text{NH}_2\text{D}$ :



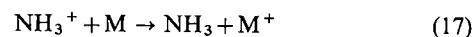
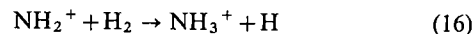
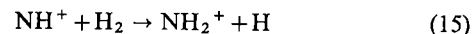
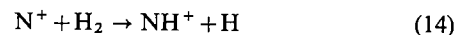
As these competing channels involve the fission of an N–H and N–D bond respectively, a very strong preference for the former is

expected in low temperature conditions owing to the substantially greater zero point energy of an N–H bond (typically the zero-point energy difference is  $\sim 800 \text{ cm}^{-1} \equiv 1,200 \text{ K}$ ). Also a similar preference is expected in the second step



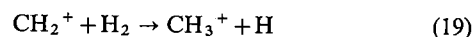
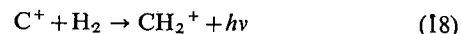
Such preferential decompositions, equations (10) and (12), lead to a DNC/HNC ratio equal to that of  $\text{NH}_2\text{D}/\text{NH}_3$ , that is, to a threefold deuterium enrichment compared with that in ammonia.

The mechanism<sup>7,13</sup> of ammonia production in dense molecular clouds is believed to be

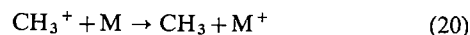


where  $\text{M}^+$  is a readily ionisable heavy atom such as Ca, Mg, Si. The formation of  $\text{NH}_2\text{D}$  therefore originates from processes analogous to equations (14), (15) and (16) involving HD. The effect of zero point energies will again be to lead to preferential retention of deuterium in the product molecular ion so that a given abundance ratio of  $\text{HD}/\text{H}_2$  could yield a ratio  $\text{NH}_2\text{D}/\text{NH}_3 = 3(\text{HD}/\text{H}_2)$ . Overall we might expect an enrichment of DNC/HNC up to three times  $\text{HD}/\text{H}_2$  corresponding to an overall sixfold enrichment of deuterium as compared with that exhibited by the dominant species in the molecular cloud ( $\text{H}_2$ ) and thus of the cloud as a whole.

For HCN we need to consider deuteration of species leading to  $\text{CH}_3^+$  or  $\text{CH}_3$ . In the case of the former the processes<sup>14</sup> are probably



which implies that the  $\text{CHD}^+/\text{CH}_2^+ = \text{HD}/\text{H}_2$  and  $\text{CH}_2\text{D}^+/\text{CH}_3^+ = 2(\text{HD}/\text{H}_2)$ . Thus if equations (18), (19), (8) and (4) show the dominant processes for HCN production we could expect an overall deuterium enrichment of HCN of up to four times that of the cloud as a whole. It is not yet clear what is the main channel of production of  $\text{CH}_3$  but if it involves



analogous to equation (17) then we expect the same degree of enrichment to arise for the equations (7) and (8). Thus in either case the expected enrichment of HCN is less than that for HNC.

If, however, HCN is predominantly produced from HNC according to equation (9) then the enrichment corresponds to that of the  $\text{D}^+/\text{H}^+$  ratio because it is this final step that introduces the proton or deuteron that ends up in the HCN or DCN. For dense clouds it is not obvious that the  $\text{D}^+/\text{H}^+$  ratio will exceed that of the overall deuterium/hydrogen ratio for the cloud and so, to the extent that HCN is produced by HNC, we expect diminished deuterium enrichment for this molecule.

While this implies that a greater enrichment of deuterium will be observed for HNC than for HCN, and this is in keeping with the limited observations so far made, it does not account for the very great enrichments, about 100-fold for HCN, 2,000-fold for HNC so far reported<sup>3,4</sup>. Since it does not seem feasible for any relevant chemical equilibria to be established on the time scales available for dense interstellar clouds we are forced to turn to kinetic isotope effects. These ion–molecule reactions, however, are all believed to proceed at rates close to the Langevin rate and this leaves little scope for isotope effects.

Perhaps the dissociative recombination reactions in equations (4) and (6) exhibit isotope effects of the requisite magnitude,

although the theory of secondary isotope effects in unimolecular reactions<sup>1,5</sup> indicates that the deuterium-containing species would decompose more slowly at the low pressure limit. It seems important to clarify this, either experimentally or theoretically. It would help if the  $\text{NH}_2\text{D}/\text{NH}_3$  ratio could be established by observations. Resolution of the problem is urgent because otherwise we must conclude either that the ion-molecule scheme of galactochemistry is inadequate or that the dense molecular clouds are enriched overall in deuterium by a handsome margin compared with the 'standard' cosmic abundance of  $\text{D}/\text{H} = 2 \times 10^{-5}$ .

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1. York, D. G. & Rogerson, J. B. *Astrophys. J.* **203**, 378 (1976).
2. Jefferts, K. B., Penzias, A. A. & Wilson, R. W. *Astrophys. J. Lett.* **179**, L57 (1973).
3. Penzias, A. A., Wannier, P. G., Wilson, R. W. & Liuko, R. A. *Astrophys. J.* **211**, 108 (1977).
4. Godfrey, P. D., Brown, R. D., Gunn, H. I., Blackman, G. L. & Storey, J. W. V. *Mon. Not. R. astr. Soc.* **180**, 83P (1977).
5. Solomon, P. M. & Woolf, N. J. *Astrophys. J. Lett.* **180**, L89 (1973).
6. Watson, W. D. *Astrophys. J. Lett.* **181**, L129 (1973).
7. Herbst, E. & Klemperer, W. *Astrophys. J.* **185**, 505 (1973).
8. Watson, W. D. *Astrophys. J. Lett.* **183**, L17 (1973).
9. Allen, M. & Robinson, G. W. *Astrophys. J.* **212**, 396 (1977).
10. Pearson, P. K. & Schaeffer, H. F. *Astrophys. J.* **192**, 33 (1974).
11. Pearson, P. K., Blackman, G. L., Schaeffer, H. F., Roos, B. & Wahigun, W. *Astrophys. J. Lett.* **184**, L19 (1973).
12. Blackman, G. L., Brown, R. D., Godfrey, P. D. & Gunn, H. I. *Chem. phys. Lett.* **34**, 241 (1975).
13. Watson, W. D. *Astrophys. J. Lett.* **188**, 35 (1974).
14. Dalgarno, A. & Oppenheimer, M. *Astrophys. J.* **192**, 597 (1974).
15. Rabinovitch, B. S. & Setser, W. *Adv. Photochem.* **3**, 1 (1964).

## Lost Pacifica continent

THE Alpine mountain chain is generally accepted to be the product of continent-continent collisions. In this belt the zone of recent tectonic activity is wide (up to 2,000 km in Tibet) and crustal thickness in places is 1.5–2 times the average continental crust, presumably due to the inability of light continental material to sink into the asthenosphere. Under the Himalayas, for example, the crust is 70 km thick<sup>1</sup>. Furthermore, as indicated by seismicity, the active collision zone here includes not only the highly deformed Himalaya belt but also the entire Tibet plateau. Major wide mountain belts exist, morphologically similar to the Alpine belt, in regions which do not experience continental collision, such as western North America, Alaska, east Siberia and the Andes. The crustal thickness here can also be very great, up to 70 km in the Andes<sup>2</sup>. All are seismically active, wide, highly deformed and include high plateaus of various sizes. Many of these wide orogenic belts also exhibit great geological complexities which are not simply explained by the model of an oceanic lithosphere under-thrusting a continental lithosphere. We suggest, therefore, that the circum Pacific mountain belts may be the result of past continental collisions, similar to those associated with the Alpine belt. We summarise the evidence for the incorporation of past continental masses around the Pacific Ocean. Holmes<sup>3</sup> has given a compelling case for large continental land masses during parts of late Palaeozoic to early Tertiary to the west of North America such as Cascadia and Llanoria<sup>4</sup>. The land includes conglomerates derived from crystalline sialic rocks which have since disappeared. Hamilton<sup>5</sup> and Davis and Armstrong<sup>6</sup> suggested that the Klamaths were originally some distance offshore to the west and that the Permo Triassic Sonoma Orogeny results from an arc continent collision.

A large scale collision of Alaska with a continental fragment during Palaeozoic and early Mesozoic, finally coalescing in late Jurassic–early Cretaceous times has been suggested<sup>7–9</sup>. Hamilton<sup>5</sup> has proposed that Permian terrains

bearing Tethyan fusulinids may have formed in the central Pacific on island arcs which were subsequently swept into the North American continent. Furthermore, these North American terrains share Jurassic and Cretaceous faunas and floras with New Zealand, Caledonia, the Antarctica peninsula and Chile. This is consistent with several palaeomagnetic studies<sup>10,11</sup> which suggest that large fragments in the western USA, Canada, and in Alaska were located near the equator perhaps at Triassic times. Hillhouse<sup>12</sup> found that tholeiitic flows in the Wrangell mountain area were formed at 15° north or south of the equator during Triassic time.

Jones *et al.*<sup>13</sup> have shown that a large continental block—Wrangellia—was incorporated in north-western North America in late Mesozoic time. This block(s)<sup>14</sup> extending over 2,000 km from Alaska to Oregon, were not contiguous to central Alaska at Jurassic time.

Most remarkably, the displaced Wrangellia block apparently received enormous quantities of Triassic tholeiitic basalts—to become one of the largest domains of non oceanic basalts<sup>13</sup>. Jones proposed that presumably, rifting initiated this volcanism, but where it occurred and what was rifted remain enigmatic.

In Central and South America there are numerous old basement inclusions within the mobile belts, some of which extend well into the Pacific Ocean itself—suggesting past continental collisions. During the Jurassic, South America was bound to the west by volcanic rocks resting on strongly folded and metamorphosed rocks off Patagonia<sup>15</sup>, Ecuador<sup>16</sup>, Peru<sup>17,18</sup> and Bolivia<sup>19</sup>. Old Precambrian continental basement fragments, of unknown origins, were recognised also in the Santa Marta mountains in Columbia, the Serrania de Baudo in Panama and Columbia, the Pacific Ocean off Peru, the Nicoya complex in Central America, and the Sierra Madre de Sur in Mexico<sup>18</sup>, among others. Palaeomagnetic data<sup>20</sup> suggest that Honduras was in the Pacific in Cretaceous times.

The situation is perhaps best summarised by James<sup>21</sup>: Jurassic volcanic rocks in southern Peru are wedged in among crystalline metamorphic rocks at least 400 Myr-old. What these remnants of ancient sialic crust are doing some 300 km west of the currently exposed geosynclinal rocks of the continental margin is unknown. These rocks could be part of the palaeozoic microcontinent that lay to the west of the South American coastline.

In north-east Asia palaeomagnetic data<sup>22</sup> suggest that the Kolyma block and the Sikhote Alin region have been welded onto the Asian continent, probably in late Jurassic or Cretaceous times. The Verkhoyansk and Sikhote mountain belts represent, therefore, probable sites of continental collisions<sup>23</sup>. Other possible continental fragments from the Pacific Ocean are the Sea of Ohotzk and surrounding areas in early Tertiary time, almost all of China south of 40°, and Korea<sup>24,25</sup>. All this may suggest that large fragments have collided with mainland Asia during Triassic through Cretaceous time. Furthermore, Churkin and Eberlein<sup>14</sup> point out that Permian rocks lie outboard of poorly known Palaeozoic and Precambrian rocks in the Alaska range. Ancient rocks reappear west of the Bering Sea along the north-west rim of the Pacific, where similar terrains of Palaeozoic age occur in north-eastern USSR, in Japan, and discontinuously further south along the west Pacific rim. Thus not unlike North America and the Andes, mysterious continental masses in the Pacific have been involved in East Siberia, China and Japan. Many of these bodies bear strong evidence for continental origins as indicated by the nature of the old rocks exposed.

We propose that these chunks were parts of a continental mass which has disaggregated, perhaps the way Gondawana has, and Africa is and may continue to disaggregate. We envisage that the circum Pacific fragments were embedded in the major plates of the Pacific Ocean—the Kula,



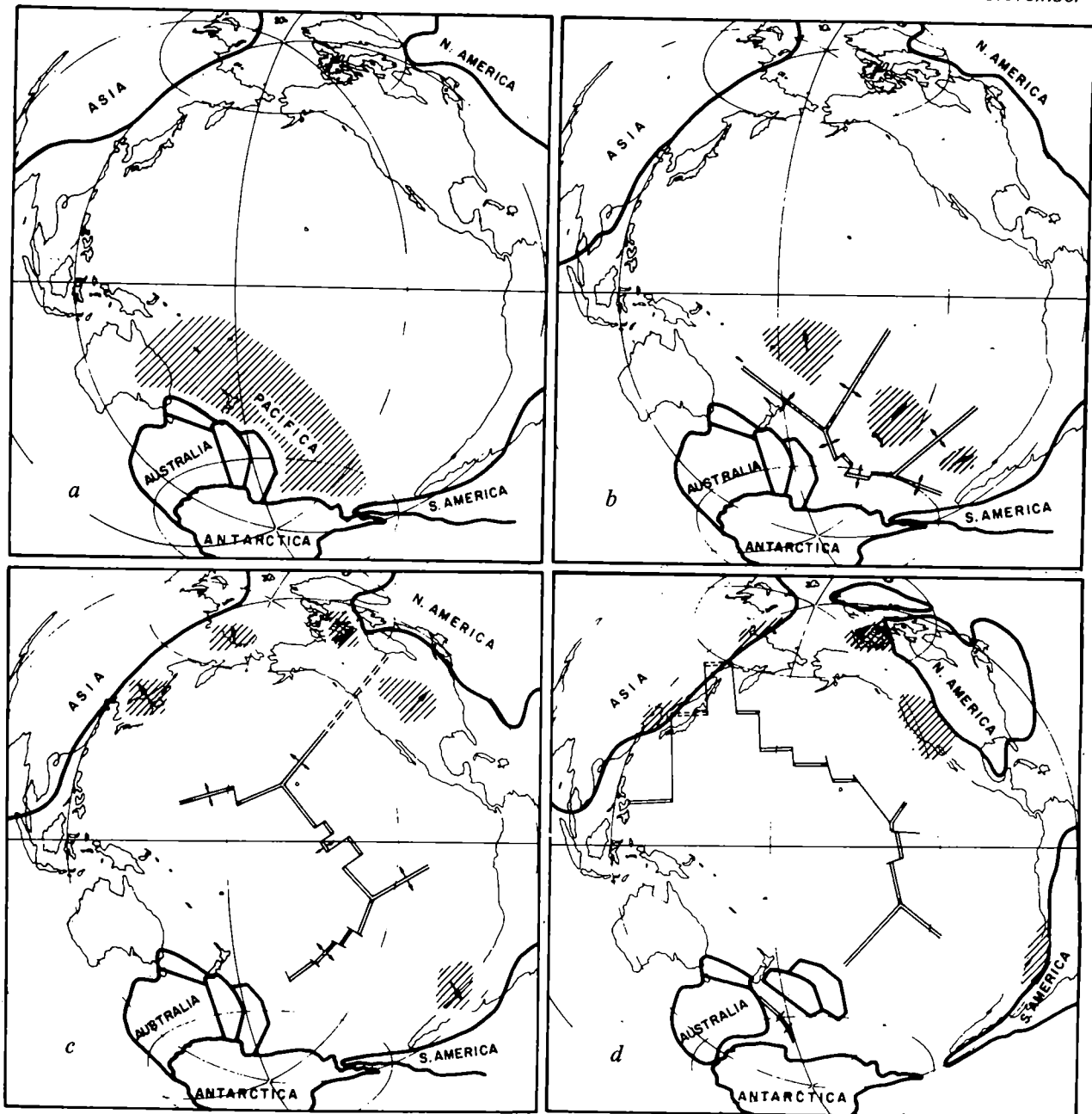


Fig. 1 Schematic model of the breakup of Pacifica and the resulting collision events. Possible ages of the reconstruction stages: *a*, 225 Myr; *b*, 180 Myr; *c*, 135 Myr; *d*, 65 Myr. Fine lines mark the present day continental outline. Heavy lines mark the location of the various continental areas through the geological evolution after Dietz and Holden<sup>39</sup>. Position of spreading centres simplified from refs 25, 26, 40–42.

Farallon, Phoenix and Pacific plates—whose motion might roughly be reconstructed from palaeomagnetic data back to 190 Myr BP<sup>25,26</sup>. As shown schematically in Fig. 1 we do this by removing continental blocks from Alaska and western North America, the Andes, Kamchatka and Japan, displacing them in a cartoon like fashion back in time attached to their corresponding plates. The various fragments, as they migrate back towards their respective spreading ridges, also approach each other. By further extrapolating the plate motions backwards beyond Jurassic time, we suggest that they comprise a single mass perhaps by mid-Permian times.

We call this mass Pacifica<sup>32</sup>—to emphasise its centrality in the Pacific geological history. Assuming that Pacifica was located over the developing spreading pattern of Larson and Chase<sup>25</sup> we imagine four major groups of continental fragments—one each on the Kula, Farallon, Phoenix and Pacific plates. As spreading continued, these fragments

were presumably carried along toward subduction zones, eventually reaching continental margins. Roughly speaking the Kula fragments collided with Alaska and Eastern Siberia, the Farallon fragments with North America and the Phoenix fragments with South America. The submerged platforms in the south-west Pacific (which show typical crustal structures) such as the Ontong-Java and Manihiki platforms may thus be fragments of Pacifica.

Before its breakup Pacifica could have been somewhere in the neighbourhood of Australia, as shown in Fig. 1. We chose to extend Pacifica to Proto Antarctica, to account for the possibility that southern South America may be a splinter off Antarctica<sup>27</sup> and that at least parts of the Antarctica coast does consist of rifted margins<sup>28</sup> indicating continental rifting.

The existence of Pacifica may thus explain the origin of the circum Pacific Cordillera, and probably shed some light on the origin of the submerged platforms in the

south-west Pacific Ocean<sup>29,30</sup>. It may provide the continental connection between western North America, south-east Asia, Australia, and South America, needed to explain the evolutionary history of flora, such as the angiosperms<sup>31,32</sup>, and fauna, such as various fusulinids<sup>3</sup> and mammals<sup>33,34</sup> around the Pacific. In fact, the concept of a Pacifica was first introduced by biogeographers<sup>32,35-37</sup> solely to explain the relation between the species and families surrounding the Pacific. Our results may, therefore, provide the geophysical and geological detail necessary to understand the continental and biological history in the Pacific. We believe that the combined evidence from geophysics, geology and biology makes a compelling case for a now extinct Pacifica continent, whose fragmented remains are mostly now embedded in the circum Pacific mountain belts.

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- Narain, H. *Tectonophysics* 20, 249 (1973).
- James, D. E. *J. geophys. Res.* 76, 3246 (1971).
- Holmes, A. *Principles of Physical Geology* (Ronald, New York, 1965).
- Schuchert, C. & Dunbar, C. O. *Outlines of Historical Geology* (Wiley, New York, 1950).
- Hamilton, W. *Geol. Soc. Am. Bull.* 80, 2409 (1969).
- Fischer, J. F. *Geotimes* 21, 18 (1976).
- Monger, J. W. H. & Ross, C. A. *Can. J. Earth Sci.* 8, 259 (1971).
- Jones, D. L., Irwin, W. P. & Ovenshine, A. T. *U.S. Geol. Surv. Prof. Paper*, 800B, B211, (1972).
- Richards, H. G. *Am. Ass. Petrol. Geol. Bull.* 58, 79 (1974).
- Symond, D. T. A. *Can. geol. Survey Paper* 71-24, 11 (1971).
- Irving, E. & Yule, R. W. *Ottawa, Earth Phys. Branch Pubs.* 42, 87 (1972).
- Hillhouse, J. *Can. J. Earth Sci.* (submitted).
- Jones, D. L., Silberling, N. J. & Hillhouse, J. *Can. J. Earth Sci.* (in the press).
- Churkin, M. & Eberlein, G. D. *Bull. geol. Soc. Am.* 88, 769 (1977).
- Cristi, J. M. *Geol. Soc. Am. Mem.* 65, 187 (1956).
- Weeks, L. G. *Am. Ass. Petrol. Geol. Bull.* 31, 1194 (1947).
- Marks, J. G. *Geol. Soc. Am. Mem.* 65, 277 (1965).
- Tschopp, H. J. *Geol. Soc. Am. Mem.* 65, 253 (1956).
- Ahlfeld, F. *Geol. Soc. Am. Mem.* 65, 171 (1965).
- Gose, W. A. & Swartz, D. G. *Geology* 5, 505 (1977).
- James, D. E. *Scient. Am.* 229, 61 (1973).
- McElhinny, M. W. *Palaeomagnetism and Plate Tectonics*, (Cambridge University Press, Cambridge 1973).
- Hamilton, W. *Geol. Soc. Am. Bull.* 81, 2553, (1970).
- Kawai, N., Hirooka, K. & Nakajima, T. *Palaeogeog. Palaeoclim. Palaeocool.* 6, 277 (1969).
- Hurley, P. M. *Eos* 52, 356, (1971).
- Larson, R. L. & Chase, C. G. *Geol. Soc. Am. Bull.* 83, 3627 (1972).
- Hilde, T. W. C., Uyeda, S. & Kroenke, L. in *Geodynamics: Progress and Prospects* (ed. Drake, C. L.) 238 (AGU, Washington, 1976).
- Windhausen, A. *Geologia Argentina* 2, (1931).
- Houtz, R. & Davey, F. J. *J. geophys. Res.* 78, 3448 (1973).
- Winterer, E. L. in *Geophysics of the Pacific Ocean and its Margin*, (eds Sutton, G. H., Manghanani, M. H. & Moberly, R.) (AGU Monograph 19, 1976).
- Nur, A. & Ben-Avraham, Z. *Eos* 58, 502 (1977).
- Melville, R. *Nature* 211, 116 (1966).
- Hughes, T. *Palaeogeog. Palaeoclim. Palaeocool.* 18, 1 (1975).
- Fooden, J. *Science* 175, 894 (1972).
- McGowan, B. *Science* 183, 759 (1973).
- Croizat, L. *Panbiogeography*, 3 vols (Croizat, Caracas, 1958).
- Martin, P. G. in *Biology of Marsupials*, (ed. Stonehouse, B.) 97-116 (University Park Press, Maryland, 1976).
- Nelson, G. *Syst. Zoology* 24, 490 (1975).
- Dietz, R. S. & Holden, J. C. *J. geophys. Res.* 75, 4939 (1970).
- Uyeda, S. & Ben-Avraham, Z. *Nature phys. Sci.* 240, 176 (1972).
- Ben-Avraham, Z. & Uyeda, S. *Earth planet. Sci. Lett.* 18, 365 (1973).
- Hayes, D. E. & Ringis, J. *Nature* 243, 454 (1973).

## Rb-Sr ages of early Archaean supracrustal rocks and Amitsoq gneisses at Isua

PREVIOUS field descriptions<sup>1-4</sup> and isotopic age determinations<sup>5,6</sup> left unresolved several important points about the relationships between the Isua supracrustal rocks and the surrounding Amitsoq gneisses. In scattered areas of comparatively low deformation, field relationships show unequivocally that

gneissose granitic veins intrude the supracrustal sequence. Geochemical studies<sup>7</sup> show that these veins are closely comparable to early tonalitic components in the polyphase Amitsoq gneisses surrounding the supracrustal belt. Here we show that the veins are isotopically indistinguishable from the main outcrops of Amitsoq gneisses. They do not represent a period of granitic activity significantly younger than the Amitsoq gneisses, such as the ~2,900 Myr-old Nûk gneisses of the Godthaab area. Thus the veins may be classified as Amitsoq gneisses. Present field and isotopic evidence combined suggest that at least part of the ~3,700 Myr-old Amitsoq gneisses are younger than the Isua supracrustal belt. We have found no field or isotopic evidence that any part of the Amitsoq gneisses so far mapped pre-date the deposition of the supracrustals.

It was also uncertain whether quartzofeldspathic gneisses so far studied isotopically from the Isua area (West Greenland) were representative of the main body of Amitsoq gneiss in that area. The earlier material studied was collected largely from gneisses close to the supracrustal rocks. Most of the samples are strongly foliated rocks in which primary igneous features had been lost during deformation. In contrast Amitsoq gneisses with original structures away from the immediate contact of the supracrustal succession were represented by only a few samples. It was thought possible that the rocks in the contact zone could represent a separate unit, either a screen of granite material emplaced along a tectonically controlled zone between an old gneiss basement and a younger cover or a group of greywacke-like sediments deposited on gneissic basement.

The material already studied isotopically<sup>5,6</sup> was supplemented by samples collected by Bridgwater and McGregor in 1973 of which the field relations were better known. Three groups of rocks were selected for further Rb-Sr age study: (1) veins of tonalitic gneiss up to several metres in width which cut across an earlier foliation in Isua amphibolitic supracrustal rocks outcropping to the west of the lake Imarssuaq (Fig. 1). These have tonalitic or granodioritic compositions comparable to the majority of Amitsoq gneisses (samples 158528-530). (2) Massive, granodioritic Amitsoq gneiss collected approximately 2.5 km north of the contact between supracrustals and gneisses east of Imarssuaq (samples 117991-993). (3) Homogeneous, muscovite-bearing, leucocratic schist found as a major unit over 100 m wide within the supracrustal succession to the west of Imarssuaq (samples 158509, 158526, 158526A). These were considered by Bridgwater and McGregor to be either acid volcanics or more highly deformed equivalents of the discordant tonalitic veins described in (1) above. Thin section and chemical studies<sup>7</sup>, however, have shown that they are closely related to K-rich boulders found within the conglomeratic unit which forms a conspicuous part of the southern and eastern parts of the Isua succession<sup>1-4</sup>. Further mapping by Allaart<sup>3,4</sup> has shown that this unit of leucocratic schist adjoins the westward continuation

Table 1 Rb-Sr analytical data

Sample no.	<sup>87</sup> Rb/ <sup>86</sup> Sr*	<sup>87</sup> Sr/ <sup>86</sup> Sr	Rb (p.p.m.)†	Sr (p.p.m.)†
Group 1				
158528	0.288	0.7165 ± 1	43	490
158529	0.310	0.7171 ± 1	22	206
158530	0.996	0.7556 ± 1	47	149
Group 2				
117991	0.787	0.7413 ± 1	91	336
117992	0.898	0.7471 ± 1	98	315
117993	0.945	0.7521 ± 1	95	294
Group 3				
158509	0.901	0.7495 ± 1	68	220
158526	5.84	1.0133 ± 2	139	71
158526A	3.36	0.8807 ± 1	93	82

\*Determined by X-ray fluorescence, average precision ± 1%.

†Mass absorption coefficients estimated from background; concentration data ~ ± 5%.

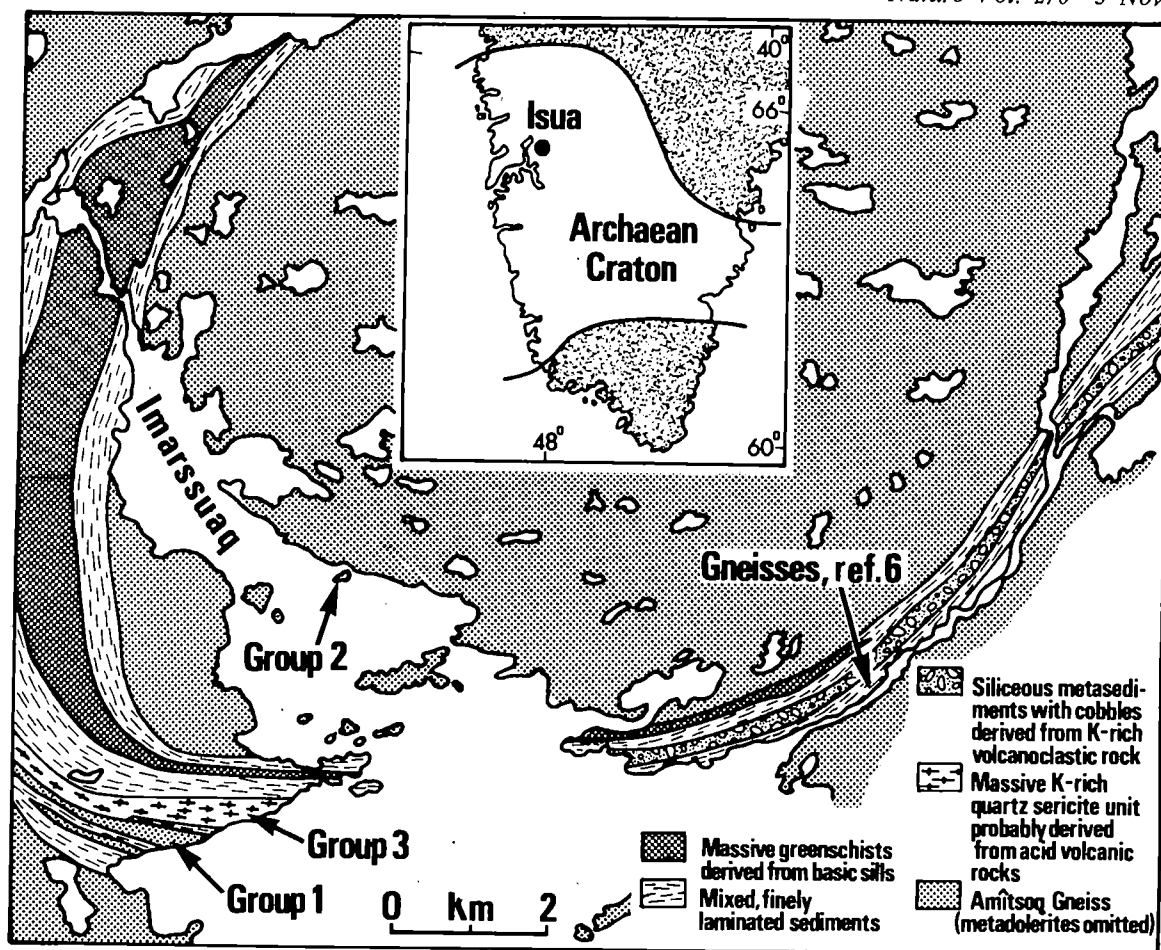
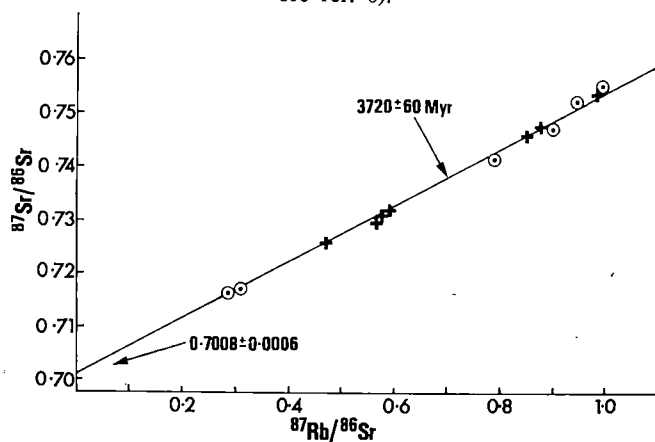


Fig. 1 Simplified geological sketch map of Isua area, after Allaart<sup>3,4</sup> with specimen localities shown.

of the conglomerate beds. The muscovite schists are now regarded as belonging to the same suite of acid, volcanogenic rocks as the conglomerate. They may be derived from acid lavas that were the source rocks for many of the boulders found in the adjacent volcanogenic conglomerate.

Table 1 shows Rb-Sr whole rock analytical data on three samples from each of the three groups listed above. Full details of chemical and mass-spectrometric techniques have been described elsewhere<sup>8</sup>. Rb/Sr ratios were determined by a precise X-ray fluorescence technique<sup>9</sup>. The decay constant of <sup>87</sup>Rb was taken as  $1.39 \times 10^{-11} \text{ yr}^{-1}$ .

Fig. 2 Rb-Sr whole rock isochron plot for Amitsoq gneisses from Isua. ○, Gneissic veins cutting supracrustals (Group 1, Table 1) and Gneisses far away from contact with supracrustals (Group 2, Table 1). +, Gneisses from near contact with supracrustals (for full details and analytical data, see ref. 6).



The groups are rather small to be treated individually but may be regressed in combination with each other and with published data. Groups 1 and 2, respectively representing gneissic veins cutting supracrustal rocks and Amitsoq gneiss well away from the contact, give a combined isochron age of  $3,750 \pm 200 \text{ Myr}$  (all errors on ages quoted at  $2\sigma$ ), and initial  $^{87}\text{Sr}/^{86}\text{Sr}$  of  $0.7008 \pm 14$  (also quoted at  $2\sigma$ ). The isochron fit is not perfect and analytical errors would have to be magnified by a factor of 3 to achieve perfect fit. When these data are regressed together with a published 7-point whole-rock isochron<sup>6</sup> of  $3,780 \pm 130 \text{ Myr}$  and initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.6998 \pm 12$  for Amitsoq gneiss from close to the margin with the supracrustal rocks, the combined data yield an isochron giving an age of  $3,720 \pm 60 \text{ Myr}$  and initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.7008 \pm 6$  (Fig. 2). This demonstrates that the gneissic veins of Group 1 and the Amitsoq gneisses were formed within a period covered by the analytical error on each rock suite. We, therefore, consider them to be contemporaneous and that the Isua supracrustals pre-date the Amitsoq gneiss.

The three samples of Group 3 yield an isochron with an age of  $3,760 \pm 70 \text{ Myr}$  and initial  $^{87}\text{Sr}/^{86}\text{Sr}$  of  $0.7014 \pm 15$ . When regressed together with five out of six published analyses<sup>6</sup> on the probably closely related (see above) boulders and matrix from the Isua conglomeratic unit<sup>3,4,7</sup>, the combined isochron gives an age of  $3,740 \pm 60 \text{ Myr}$  and initial  $^{87}\text{Sr}/^{86}\text{Sr}$  of  $0.7015 \pm 17$  (Fig. 3). (The omitted sample from ref. 6 is 158538.) The six published analyses gave an errorchron of  $3,860 \pm 240 \text{ Myr}$ , with an intercept of  $0.675 \pm 40$ . The far more precise results reported here are due to the much greater range of Rb/Sr ratios for the combined data, as well as the omission of one of the published values. The revised age agrees well with a Pb-Pb isochron age of  $3,760 \pm 70 \text{ Myr}$  for the banded iron formation member of the Isua supracrustal sequence<sup>5</sup>.

The following principal conclusions may be drawn from these data. First, gneissic veins which intrude the Isua supracrustal sequence yield Rb–Sr whole rock ages indistinguishable from the surrounding Amitsoq gneisses. Together with fragments of greenstone belt-type lithologies (Akilia association) enclosed in Amitsoq gneisses elsewhere in the Godthaab district<sup>10</sup> the Isua supracrustals are the oldest rocks recognised in the Archaean of West Greenland.

Second, the Isua supracrustals and surrounding Amitsoq gneisses are all between ~3,700 and 3,800-Myr-old, although the actual age difference between the two rock units is probably too small to resolve by Rb–Sr and Pb–Pb whole-rock age methods. U–Pb methods on zircons may offer more hope in this respect<sup>11,12</sup>. Thus, a U–Pb zircon date of  $3,600 \pm 50$  Myr has been reported on Amitsoq gneisses from the Godthaab area<sup>11</sup>, whilst a U–Pb zircon date of  $3,824 (+12 \text{ or } -9)$  Myr has been reported from metavolcanic boulders in a large conglomeratic unit within the Isua supracrustals<sup>12</sup>. Unfortunately, no zircon U–Pb dates have yet been published for the Amitsoq gneisses at Isua.

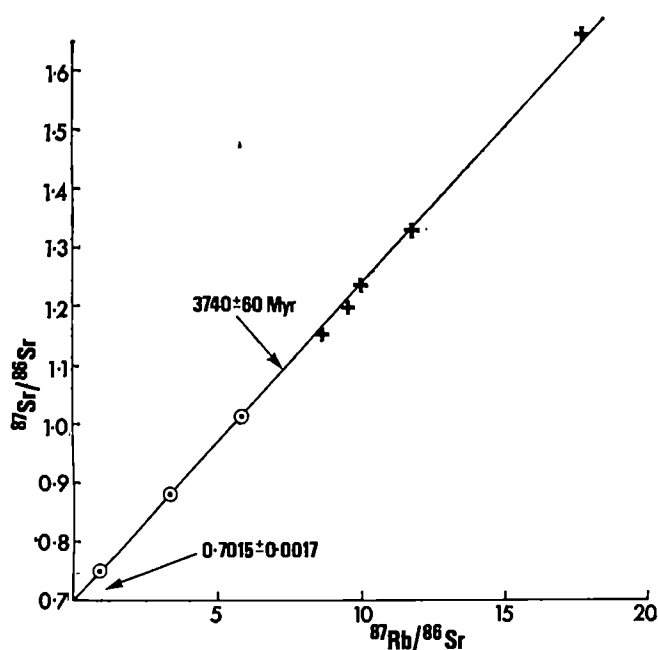


Fig. 3 Rb–Sr whole rock isochron plot for Isua supracrustal rocks. ○, Schists (Group 3, Table 1). ×, boulders and matrix from conglomeratic unit (for full details and analytical data, see ref. 6).

Finally, initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are in the range ~0.700–0.702 and suggest, in agreement with previous views<sup>13,14</sup>, that the precursors of these early Archaean rock units had not existed with average crustal Rb/Sr ratio for more than about 100 Myr before the measured age.

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1. Bridgwater, D. & McGregor, V. R. *Geol. Surv. Greenland*, Rep. no 65, 49 (1974).
2. Bridgwater, D., Keto, L., McGregor, V. R. & Myers, J. S. in *Geology of Greenland* (eds. Escher, A. & Watt, W. S.) (Geol. Surv. Greenland, Copenhagen) 1976).

3. Allaart, J. H. *Geol. Surv. Greenland*, Rep. no 75, 53 (1975).
4. Allaart, J. H. in *The Early History of the Earth* (ed. Windley, B. F.) (Wiley, London, 1976).
5. Moorbath, S., O'Nions, R. K. & Pankhurst, R. J. *Nature* 245, 138 (1973).
6. Moorbath, S., O'Nions, R. K. & Pankhurst, R. J. *Earth planet. Sci. Lett.* 27, 229 (1975).
7. Bridgwater, D., Collerson, K. D. & Myers, J. S. in the *Evolution of the Earth's Crust* (ed. Tarling, D. H.) (Academic, London, in the press).
8. O'Nions, R. K. & Pankhurst, R. J. *Earth planet. Sci. Lett.* 21, 12 (1973).
9. Pankhurst, R. J. & O'Nions, R. K. *Chem. Geol.* 12, 127 (1973).
10. McGregor, V. R. & Mason *Contrib. Miner. Petrol.* (in the press).
11. Baadsgaard, H. *Earth planet. Sci. Lett.* 33, 261 (1973).
12. Michard-Vitrac, A., Lancelot, J., Allègre, C. J. & Moorbath, S. *Earth planet. Sci. Lett.* 35, 449 (1977).
13. Moorbath, S., O'Nions, R. K., Pankhurst, R. J., Gale, N. H. & McGregor, V. R. *Nature phys. Sci.* 240, 78 (1972).
14. Moorbath, S. *Nature* 254, 395 (1975).

## Laser speckle photography in a fluid medium

A TECHNIQUE for obtaining quantitative velocity data from hydrodynamic flow fields using laser speckle photography (LSP) has been developed and uses the scattered light from the interior of a suitably seeded liquid which is illuminated by a coherent beam from a pulsed ruby laser. The resulting speckle pattern can be photographed on high resolution film. A doubly exposed photograph of the correlated speckle patterns produced by fluid dynamic motion contains all the information necessary to describe the motion throughout a selected plane. When the speckle photographs are optically interrogated distinct fringe patterns are produced whose geometries are related to the velocity field. Here we describe how a Poiseuille flow was used to demonstrate this novel technique. Doubly exposed speckle photographs and typical fringe patterns illustrating the velocity distribution are given, analysed and compared with the classical theory.

The LSP technique has been used since 1969 almost exclusively for various applications to surface metrology<sup>1–3</sup>. Barker and Fournay<sup>4</sup> have extended the method to measure displacements in the interior of a transparent solid by recording side-scattered light from an illuminated domain. With lasers of conventional power such recordings require very long exposures. Consequently, the few applications reported so far have examined only imposed static strains. The basic difference between that work and the present application to fluids is that the latter is truly dynamic involving moving liquids rather than static solids.

The extension of side-scattering speckle techniques to fluid dynamic situations has been achieved by using a double pulsed Q-switched ruby laser and a suitably seeded medium. The experimental arrangement used to record speckle photographs of the flow field is shown in Fig. 1. The beam from a ruby laser, which

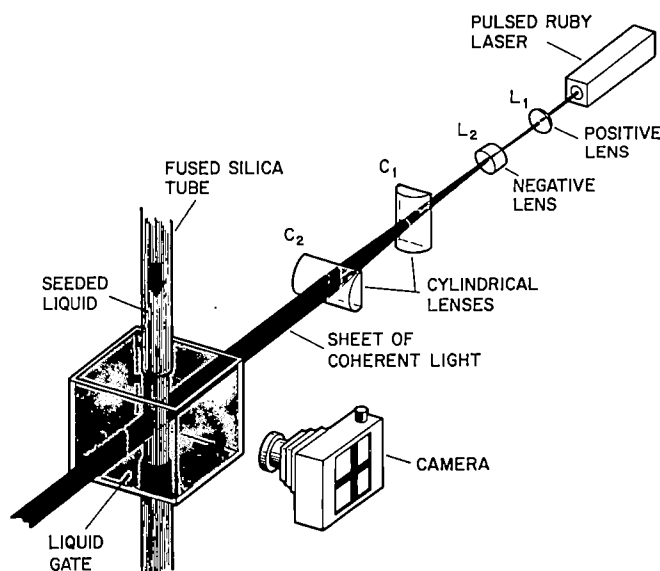


Fig. 1 Experimental arrangement for side-scattering LSP.

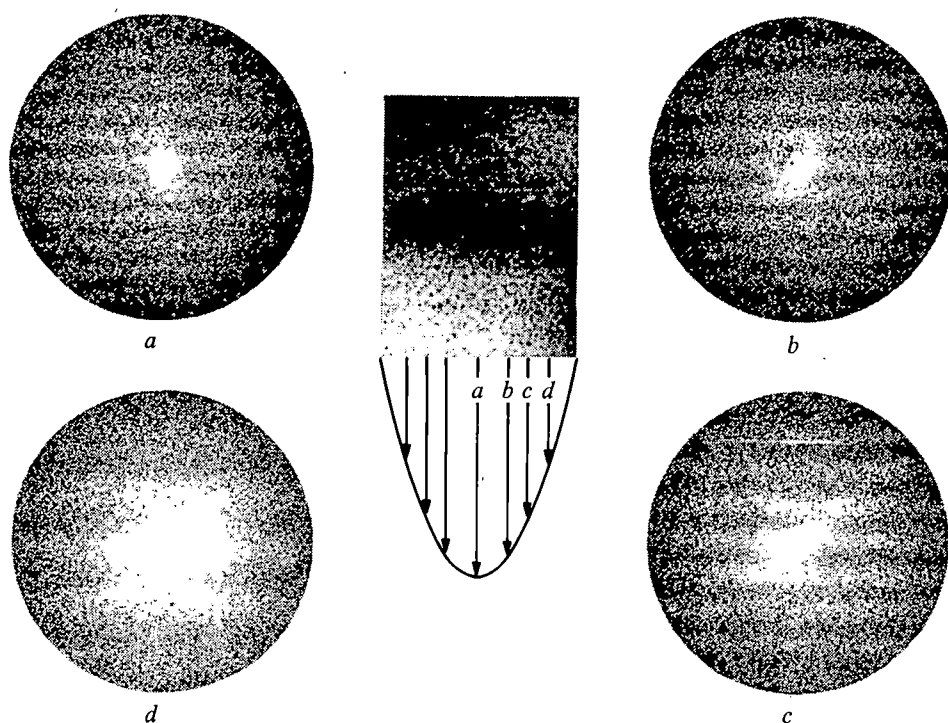


Fig. 2 Typical laser speckle photograph and diffraction fringe patterns from radial locations as shown; *a*,  $r/R = 0$ ; *b*,  $r/R = 0.32$ ; *c*,  $r/R = 0.53$ ; *d*,  $r/R = 0.74$ .

delivers 2–4 J in each 20-ns pulse, is condensed by lens  $L_1$ . A negative lens  $L_2$  is placed ahead of the focal point of  $L_1$ . From the virtual focus of  $L_2$  the beam is expanded and collimated into a vertical planar sheet about 40 mm high and 1–1.5 mm thick by the cylindrical lenses  $C_1$  and  $C_2$ . A slit may be inserted at the observation cell to assure a beam of uniform 1 mm thickness, but is not essential. The above arrangement of optical components specifies the interior plane of study and optimises the amount of light available to be side-scattered from the flow field.

To demonstrate the technique, a Poiseuille flow was established in a quartz tube using glycerine seeded with a minute quantity of

white latex paint ( $\sim 0.01$  ml per l). Refraction effects due to the curvature of the tube were minimised by enclosing the illuminated portion in a liquid gate filled with an index matching fluid. The correlated speckle patterns were recorded on Agfa-Gevaert 10E75 high resolution film. Double-exposed speckle photographs, with time separations of 10–15 ms were recorded at a magnification  $m$  of 2.1 and lens apertures between  $f/2.8$  and  $f/4$ . At the ruby wavelength  $\lambda = 694.3$  nm, the Rayleigh resolution criterion

$$s_0 = \frac{1.2 \lambda f (1+m)}{m} \quad (1)$$

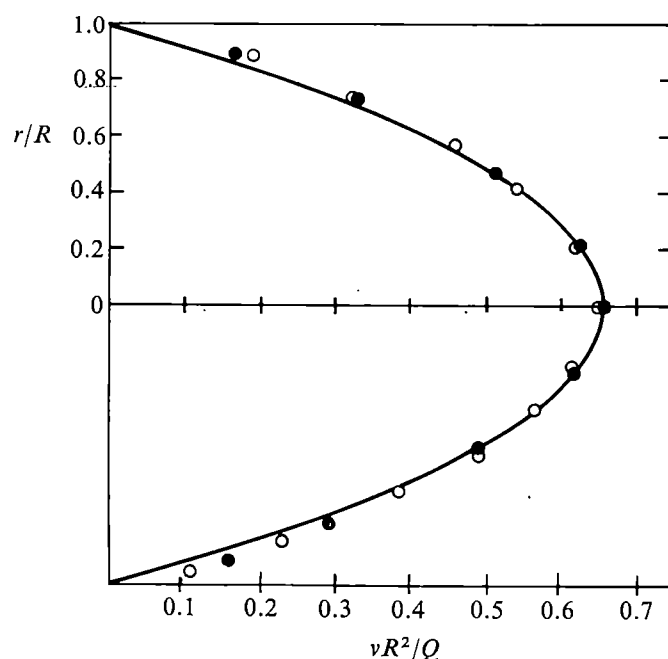
estimates the speckle size in the object plane to be  $3.4 \mu\text{m}$  and  $4.9 \mu\text{m}$  for the apertures quoted above. These speckles form a very fine random grid which moves with the medium in the illuminated plane. Therefore, a double-exposed photograph of the side-scattered light contains two correlated grids which can be analysed as a non-uniform diffraction grating. This technique can be used successfully only where the displacement between exposures is greater than the speckle diameter, but not so great as to destroy correlation. In this study, which included more than the two examples discussed here, the displacements that were most readily resolved ranged between 2 and 20 speckle diameters.

Figure 2 shows a typical double-exposed speckle photograph, and several diffraction fringe patterns which were obtained by interrogating it at various radii across a diameter of the tube. The interrogation was accomplished by illuminating the speckle photograph with a narrow beam of coherent light which diffracts into a pattern of Young's fringes. The spacing and orientation of the fringes are related to the displacement field at the point of interrogation. Specifically, the displacement of the medium,  $d$ , is related to the angular spacing of the fringes,  $\alpha$ , and the magnification,  $m$ , by

$$\sin \alpha = \lambda/dm \quad (2)$$

where  $\lambda$  is the wavelength of the interrogating beam. In addition, the displacement vector in the illuminated plane is always normal to the direction of the diffraction fringes at each point. Because Poiseuille flow is steady and unidirectional, the fringes are

Fig. 3 Comparison between the LSP data and the classical Poiseuille theory.  $\circ$ ,  $Q = 0.53 \text{ ml s}^{-1}$ ,  $\Delta t = 10 \text{ ms}$ ;  $\bullet$ ,  $Q = 0.264 \text{ ml s}^{-1}$ ,  $\Delta t = 10 \text{ ms}$ .





everywhere perpendicular to the tube axis and the spacing varies continuously and identically across any diameter. In general, in flow-fields which are not unidirectional the fringe patterns will be less regular.

Analysis of a series of diffraction patterns across a diameter gives the fluid displacement as a function of radius,  $r$ . Thus the velocity distribution can be obtained directly since the pulse separation is known. Figure 3 gives the velocity,  $v$ , for two time separations and flow rates,  $Q$ , compared with the classical distribution

$$v(r) = \frac{2Q}{\pi R^2} \left( 1 - \frac{r^2}{R^2} \right) \quad (3)$$

where  $R$  is the radius at the wall. The agreement between the LSP data and the classical theory is very good over most of the tube diameter. Near the walls, however, positional errors due to refraction are apparent because of the 0.7% refractive index mismatch between the fused silica and the glycerine.

The side-scattered LSP technique therefore offers an attractive alternative to the existing methods of studying fluid dynamic flow fields. In contrast with conventional interferometric techniques, side-scattered LSP measures the velocity field explicitly, and can examine a uniquely defined interior plane rather than integrating the results through the entire volume thickness.

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1. Archbold, E. *et al.* *Nature* **222**, 263-265 (1969).
2. Leendertz, J. A. J. *Phys. E*, **3**, 214-218 (1970).
3. Dainty, J. C. *Laser Speckle and Related Phenomena* (Springer, Berlin, 1975).
4. Barker, D. B. & Fourny, M. J. *Exp. Mech.* **16**, 209-214 (1976).

## Mean residence times in continuous flow systems

INDIVIDUAL elements of a material which enters a system at a constant rate, resides for some time, and then leaves, can take different paths through the system, travel at different speeds, and be delayed for many reasons. For example, by diffusing into stagnant zones, or by transferring, perhaps across a membrane, into a separate phase, they may take part in any activity that does not destroy them which does not preclude chemical reaction where atoms of flow material (which may be combined in any way with other components in the flow streams) are being considered. The process must, however, be a steady-state one, which implies that the quantities of material or 'holdups' in all parts of the system do not vary with time, and that the rate at which material leaves the system is equal to its rate of entry. As an example, we could consider a stretch of river swept by water, or an effluent component in the water; or a continuous chemical reactor into and out of which a conserved material (atoms or ions perhaps) flows at a constant rate; or a respiratory system, fed with oxygen which is transported to the various parts of the body before leaving in part as carbon dioxide. At first sight it seems unlikely that quantitative deductions of the behaviour of so abstract a structure are possible; however, one such deduction has been made<sup>1</sup>, and this letter reports another.

The residence time distribution,  $f(t)$ , of the material component considered, completely defines the general system: thus, the fraction of key material leaving the system, that has resided in the system for a period of time in the interval  $(t, t+dt)$ , is  $f(t)dt$ . Buffham<sup>1</sup> has generalised a well established result for simple flow systems, showing that the mean residence

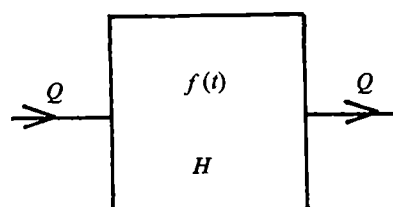


Fig. 1 Block diagram for the general system: the steady flow rate of the key material component is  $Q$  and its residence time distribution is  $f(t)$ ; its total holdup in the system is  $H$ .

time of material,  $\mu$ , is independent of the internal characteristics and always equal to the ratio of total holdup of the material to its overall flow rate through the system. Figure 1 and equation (1) summarise this.

$$\mu = \int_0^\infty t f(t) dt = H/Q \quad (1)$$

Obvious uses of this are in the measurement of the holdup of the component, or its overall flow rate, where the residence time distribution can be determined by tracer techniques<sup>2</sup>, but its usefulness goes well beyond these direct applications. For example, it has been central to the development of a model-independent theory of chromatography<sup>1</sup> which supercedes previous treatments, based on specific models of the chromatographic process, by obviating the restrictive assumptions on which those are founded.

We shall now use the basic relationship shown in equation (1) to make deductions about arbitrarily defined internal regions of the general system; a more complete proof of the final result, equation (2), will be published elsewhere.

Consider the general system divided into  $n$  regions having holdups of the key flow material  $h_1, h_2, \dots, h_n$  where

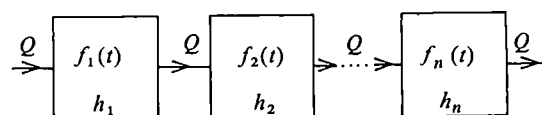
$$\sum_i h_i = H$$

The residence time distributions of the material in those regions is denoted by  $f_1(t), f_2(t), \dots, f_n(t)$  respectively. In general, an element may enter a region any number of times; however, periods of residence that make up these distributions refer to total times spent by elements in the respective regions before they leave the system. The period of residence of a material element in the system will be the sum of its residence times in the  $n$  regions. Assuming these separate periods of residence to be independent of each other (and it is difficult to think of a steady-state system where this condition is not satisfied), then the distribution of the sum of residence times in the various regions,  $f(t)$ , may be obtained by convolution<sup>3</sup>. Thus the  $n$ -fold convolution

$$f(t) = \int_0^t \int_0^{\lambda_1} \dots \int_0^{\lambda_{n-2}} f_n(t - \lambda_1) f_{n-1}(\lambda_1 - \lambda_2) \dots f_2(\lambda_{n-2} - \lambda_{n-1}) f_1(\lambda_{n-1}) d\lambda_{n-1} d\lambda_{n-2} \dots d\lambda_1$$

gives the total residence time distribution. This relationship is represented by Fig. 2 which is thus equivalent to Fig. 1.

Fig. 2 Block diagram of the general system that is equivalent, for residence time distribution purposes, to Fig. 1.



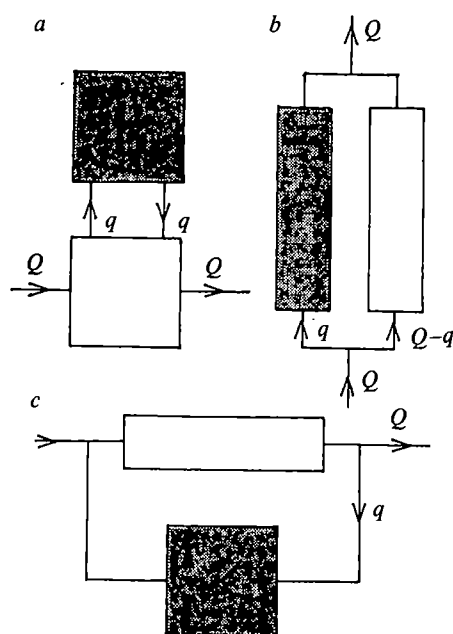


Fig. 3 Examples of simple continuous flow systems for which the general relationship of equation (2) may be readily verified for the shaded regions. Squares and rectangles represent perfectly mixed and plug-flow regions respectively.

Equation (1) may now be applied to any of the blocks of Fig. 2 with the result

$$\mu_i = \int_0^{\infty} t f_i(t) dt = h_i/Q \quad (2)$$

$i = 1, 2, \dots, n$

which states that the mean residence time of material in any region of the system is equal to its holdup in that region divided by its overall flow rate through the system as a whole.

This simple result is rather surprising. Figure 3 shows some trivial examples for which it may be readily verified for the shaded regions: although in each case, the residence time distribution of flow material in this region depends on the internal flow rate,  $q$ , the mean residence time does not and is given by equation (2).

These examples cease to be trivial if arbitrary mixing patterns are allowed in the regions as well as additional connections between them. Hence, Fig. 3a and b may well model the stretch of river referred to earlier with the shaded areas representing a relatively stagnant backwater; regardless of the descriptive complexities of such a system, equation (2) will hold for both regions and could be applied, say, to making a first estimate of the consequences of an upstream effluent discharge on the ecology of the backwater. Another application could be in predicting the exposure of various regions of a system to a radioactive tracer introduced, for diagnostic purposes, into a flow stream to which they may be only indirectly connected: numerous systems, including physiological ones, are frequently tested in this way.

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1. Buffham, B. A. *Proc. R. Soc. Lond. A* 333, 89–98 (1973).
2. Danckwerts, P. V. *Chem. Engng. Sci.* 2, 1–13 (1953).
3. Kendall, M. G. & Stuart, A. *The Advanced Theory of Statistics 1* (Charles Griffin, London, 1969).

## *Centroceras*, the 'missile'-launching marine red alga

VEGETATIVE reproduction by specialised structures is very rare in the red algae. Dixon<sup>1</sup> has listed two cases among freshwater species and two unconfirmed reports from the nineteenth century among marine species. I report here that the marine red alga *Centroceras* produces missile-shaped propagules which are carried away by ocean currents.

Although recorded only six times since its discovery in the Red Sea in 1849<sup>2–5</sup>, *Centroceras* seems to be quite common in the epiphytic community of the leaf surfaces of seagrasses, and to a lesser extent, larger attached algae. It is, however, inconspicuous due to intensive grazing by the many herbivorous fish of the region.

'Clean-looking' *Halophila stipulacea* (Forssk.) Aschers, plants were cultured in the laboratory. Undisturbed by fish, the epiphytic algal rudiments attached to the leaves developed within 4 d to give a luxuriant growth clearly visible to the naked eye. Among the algae isolated from this growth was *Centroceras*. (The Red Sea populations have usually been referred to as *C. clavulatum* (C. Ag.) Mont.<sup>3,6–8</sup> or *Ceramium clavulatum*<sup>4,5</sup>.) Our *Centroceras* plants differed from other *C. clavulatum* populations in showing: (1) two branching patterns at the apex regions (Fig. 1); (2) limited growth of lateral branches while attached to the parent plants; (3) unpigmented cortical cells of the lowermost internode of the lateral branches; (4) rhizoids just above the first node of the lateral branch, and (5) missile-shaped lateral branches that dropped off and were carried away as propagules.

When they reached the bottom of the culture dish, the propagules resumed growth, attached themselves to the

Fig. 1 Monopodial unilateral branching of 'missile'-bearing branch of *Centroceras* from the Red Sea. Scale bar, 250  $\mu$ m.



substrate by rapid elongation of their rhizoids and elongated rapidly. Within a few days, sometimes as soon as 4 d, the developing plants were large enough (7–10 mm long) to produce 'missiles' of their own. A great many such 'missiles' were produced by one parent plant.

Attempts to find 'missile'-bearing plants of *Centroceras* in *Halophila* beds failed as no *Centroceras* growth could be detected on the leaves. But it was found on leaves of another seagrass, *Thalassodendron ciliatum* (Forssk.) den Hartog. Unlike *Halophila*, *T. ciliatum* has leaves which are largely horizontally orientated, often forming a close canopy which prevents fish from reaching the lower leaves and facilitates undisturbed development of epiphytic algal growth.

In the sea the numerous tiny 'missiles' produced by *Centroceras* plants which escape the teeth of fish, would be carried away easily by currents and spread over large areas, to settle on leaves of seagrasses and other suitable surfaces. They serve both as long-range dispersal units and as a means to ensure quick development of the future plant which in a very short time produces new dispersal units, before falling prey to grazing fish. There are many species of herbivorous fish in tropical seas, and it may be assumed that the production of the missile-shaped propagules by *Centroceras* in the Red Sea and probably in other areas of the Indopacific region, evolved as a result of heavy grazing pressure.

In that case, other delicate algae of similar habitats there and in other tropical regions can be expected to have reacted to grazing pressure by producing specialised vegetative reproductive structures yet to be discovered.

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1. Dixon P. S. *Biology of the Rhodophyta* (Edinburgh, 1973).
2. Ruprecht, F. J. *Zap. imp. Akad. Nauk. Series 6*, 6 (Bot.) 74–84 (1849).
3. Zanardini, G. *Mem. Ist. Venet. Sci. Lett. Arti*, 7(2), 209–309 (1858).
4. Reinbold, T. *Hedwigia* 42, 227–232 (1903).
5. Muschler, R. *Mem. Inst. Egyptien* 5, 141–237 (1908).
6. Rayss, T. *Bull. Sea Fish. Res. Stn Haifa* 9, 1–36 (1959).
7. Papenfuss, G. F. *Israel J. Bot.* 17, 1–118 (1968).
8. Lipkin, Y. *Israel J. Zool.* 21, 405–446 (1972).

## Phytochrome-regulated organogenesis in lettuce tissue culture

LIGHT intensity and daily photoperiod are known to affect organ development in tissue culture<sup>1</sup>, but little information is available on the spectral region influencing organogenesis. Studies with tobacco callus have shown that shoot initiation is enhanced by blue light<sup>2,3</sup> and that red light has no influence. Red light, on the other hand, promotes callus growth and adventitious bud formation in some species<sup>4,5</sup> and induces the elongation of endogenous buds in cultured root segments of *Convolvulus arvensis* L.<sup>6</sup>. The latter phenomenon is regulated by phytochrome<sup>6</sup>. In the culture conditions described here, lettuce cotyledon cultures develop well-formed shoots under daily (16-h photoperiod) irradiation from broad-band emitting fluorescent lamps. Dark-grown control cultures, on the other hand, form significantly fewer shoots. We describe here the enhancement of shoot formation in lettuce cotyledon cultures by red light and demonstrate that organogenesis is under phytochrome control<sup>7</sup>.

Explants (3 × 2 mm) from the distal regions of the cotyledons were obtained from dark-grown, 5-d-old germinated seeds of *Lactuca sativa* var. 'Black Seeded Simpson'. Excision and planting were done under low intensity (~4.5 μW cm<sup>-2</sup>) 504-nm light to which the cultures are not sensitive. The explants were grown on MS salts<sup>8</sup> supplemented with indole-3-acetic acid 1 mg l<sup>-1</sup>, kinetin 0.5 mg l<sup>-1</sup>,

**Table 1** Red/far-red reversible effects on shoot formation and callus growth in lettuce cotyledon cultures

Treatment	Sample size	No. of shoots per culture	Callus weight (g) per culture
Expt 1			
Control (no light)	55	6.34 ± 0.56	2.25 ± 0.21
5 min R per d	30	16.96 ± 0.78*	3.25 ± 0.23†
5 min R + 5 Min FR per d	27	6.48 ± 0.92	1.70 ± 0.32
Expt 2			
Control (no light)	85	3.02 ± 0.18	2.76 ± 0.19
5 min R per d	29	6.21 ± 0.47*	4.15 ± 0.24§
5 min R + 5 Min FR per d	28	4.04 ± 0.39†	2.20 ± 0.29
Expt 3			
Control (no light)	29	3.69 ± 0.35	3.01 ± 0.23
5 min FR per d	25	3.80 ± 0.33	2.26 ± 0.20

Lettuce cotyledon cultures were exposed to the indicated conditions for 35 d before data collection. R, 240 μW cm<sup>-2</sup> 660 nm light; FR, 680 μW cm<sup>-2</sup> 740 nm light. Errors represent ± 1 s.e.m. Statistical significances given in comparison with control values.

\*0.001 ≥ P (Smirnov test).

†0.05 ≥ P > 0.01 (Smirnov test).

‡0.01 ≥ P > 0.001 (t-test).

§0.001 ≥ P (t-test).

||0.05 ≥ P > 0.01 (t-test).

adenine sulphate 40 mg l<sup>-1</sup>, thiamine-HCl 0.1 mg l<sup>-1</sup>, nicotinic acid 0.5 mg l<sup>-1</sup>, pyridoxine-HCl 0.1 mg l<sup>-1</sup>, glycine 2 mg l<sup>-1</sup>, myo-inositol 100 mg l<sup>-1</sup> and sucrose 30 g l<sup>-1</sup> (ref. 9). Before addition of Bacto-Agar (Difco) 10 g l<sup>-1</sup>, the medium was adjusted to pH 5.8. The 660-nm red light source was described previously<sup>3</sup>. The far-red source was a narrow-band emitting fluorescent lamp with peak emission at 744 nm (Phosphor E-5346, GTE) from which light below 700 nm was absorbed by FRF-700 plexiglass filter (Westlakes Plastics).

Table 1 shows the results of 5-min daily exposures of lettuce cotyledon cultures to 660 nm, 740 nm, or 660 nm followed immediately by 740 nm light. After 35 d, red light caused a 2- to 2.5-fold increase in the number of shoots and a 1.5-fold increase in callus weight compared with control cultures not exposed to light. Far-red light had no effect on the number of shoots produced but decreased the callus weight slightly. Red followed by far-red light reversed the red light effect on both shoot formation and callus growth. These results demonstrate that both increased shoot formation and callus growth are regulated by the low-energy phytochrome photoreceptor system.

The above data do not indicate at what stage of shoot development red light is effective. For example, light may simply cause elongation of shoots from pre-existing buds as is the case with *Convolvulus arvensis*<sup>6</sup>. To examine this point, both bud and shoot formation were observed at an earlier stage of development. Table 2 shows that red light

**Table 2** Red light-induced bud and shoot formation in lettuce cotyledon cultures after 14 d of exposure

Treatment	Sample size	No. of buds	No. of shoots
Expt 1			
Control	30	11.23 ± 0.74	1.47 ± 0.22
5 min R per d	26	20.34 ± 1.14	3.00 ± 0.34
Expt 2			
Control	7	10.00 ± 0.82	1.29 ± 0.52
5 min R per d	23	20.83 ± 1.29	3.09 ± 0.42

Conditions were as in Table 1.

doubles the number of buds as well as shoots present in the cultures after only 14 d of exposure. Thus, light regulation of shoot production occurs at the bud stage of development and perhaps at the bud initiation stage.

To our knowledge this report is the first demonstration of phytochrome-regulated shoot morphogenesis in a tissue culture system. Lettuce cotyledon cultures, then, may serve as a useful, simple, model system for investigating the molecular mechanisms involved in phytochrome regulation of organogenesis.

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1. Murashige, T. A. *Rev. Pl. Physiol.* **25**, 135-166 (1974).
2. Weis, J. S. & Jaffe, M. J. *Physiol. Pl.* **22**, 171-176 (1969).
3. Seibert, M., Wetherbee, P. J. & Job, D. D. *Pl. Physiol.* **56**, 130-139 (1975).
4. Ward, H. B. & Vance, B. D. *J. exp. Bot.* **19**, 119-124 (1968).
5. Kadkade, P. G. & O'Connor, H. J. *Tappl. Conf. Papers book*, 71-75 (1977).
6. Bonnett, H. T. *Planta (Berl.)* **106**, 325-330 (1972).
7. Kadkade, P. G. & Seibert, M. *Abstr. 7th int. Congr. Photobiol.*, Rome 1, 250 (1976).
8. Murashige, T. & Skoog, F. *Physiol. Pl.* **15**, 473-497 (1962).
9. Kadkade, P. G. & O'Connor, H. J. *Pl. Physiol.* **57**(5), Suppl. 75 (1976).

## Graded localisation of naming from electrical stimulation mapping of left cerebral cortex

APPLYING a weak electric current to the cerebral cortex of awake neurosurgical patients is an established method for mapping language zones on the basis of stimulation-evoked errors. Two new findings are reported here—the topographical extent of language cortex in an individual subject can be wider than that proposed in the classic maps, and, within this zone different sites are variably committed to language as measured by the naming function.

Naming errors evoked by electrical stimulation of the brain in the presence of a retained ability to speak are useful indicators of the language function of focal areas of cortex. Weak electric currents at levels below that which produce after-discharges, deactivate a limited area of cortex abruptly and reversibly, not allowing the brain sufficient time to either compensate or reorganise. Furthermore, research on aphasia has established that lesions to any part of the language system will cause anomia, or naming errors.

In the most extensive studies of stimulation mapping of the cerebral cortex, Penfield and Roberts<sup>1</sup> reported aphasic-like errors from the classical left hemisphere language zones; these errors included distortion and repetition of syllables and words, confusion of numbers while counting, inability to name with retained ability to speak, misnaming and perseveration. What is not generally recognised, however, is that Penfield and Roberts only elicited a total of 132 aphasic errors in 110 patients; of these errors, 19 were misnamings and 51 were omissions with retained ability to speak, the two error categories most representative of anomia.

More recently, Fedio and Van Buren<sup>2</sup> recorded naming errors from left hemisphere stimulation in 19 patients; in their series, a total of 87 naming errors, primarily omissions, were observed, again clustering in the classic language zones. The small number of sites sampled in any one patient again precluded analysis of the extent and the variability of language representation in the lateral cortex of the individual.

In this report we address two related questions. First, the Penfield and Roberts composite map, derived from pooling the stimulation-error points across subjects, covers a large area of the left hemisphere; is any single individual's language cortex this extensive? Second, are specific areas of cortex within the zone equally involved in language function in a particular individual?

Data bearing on these questions were obtained from three adults with psychomotor seizures from left temporal or left temporo-parietal foci of early childhood onset. The persistence of the seizures despite anticonvulsant therapy indicated surgical treat-

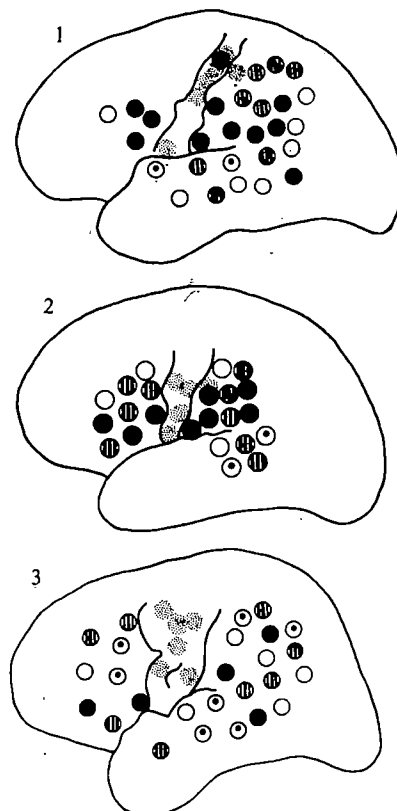
ment. Pre-operative intracarotid sodium Amytal testing, which measured naming, reading and short-term verbal memory, identified the left hemisphere as the dominant one for language in all three patients.

The left fronto-temporo-parietal cortex was exposed at operation under local anaesthesia. Corticography was used to locate the epileptic focus; after that motor and sensory responses to stimulation mapped the Rolandic cortex, which is indicated by stippled circles on the left hemisphere tracing in Fig. 1. Language testing followed, using a set of achromatic slides, each one a line drawing of a different common object such as a bell, a hand, a car, a ring, with the words 'That is a', or 'This is a', printed above. The slides were projected at a constant rate, 4 or 5 s depending on each patient's ease of responding in the test-operative situation. The patient was required to say the words, for example 'This is a', and then to name the object. Testing was carried out in the patient's native language: English for number 1 and no. 3, Dutch for no. 2.

Stimulation was delivered through bipolar silver ball electrodes, 5 mm apart. The stimulations were bi-phasic square wave pulses, 2.5 ms total width at 60 Hz, delivered from a Nuclear Chicago constant current stimulator. The current levels were determined for each patient to be below the threshold for after-discharges in epileptic cortex and below the level of sensation in sensory-motor cortex. The current level for patient 1 was 7 mA in all sites, for patient 2, 8 mA in all sites. For patient 3 it was necessary to use a lower current in the epileptic cortex, 3 mA in the anterior temporal lobe and 4 mA in the posterior temporal lobe; 6 mA were used in the frontal and parietal lobes. Current was delivered in stimulation trains of the same duration as the naming slide presentations, initiated and terminated when the slide projector changed.

A grid pattern of sterile numbered tickets was laid out on the

**Fig. 1** Results from naming error tests depicted on left hemisphere angiogram tracings. Solid circles indicate points at which stimulation always produced a naming error in that patient. Shaded circles, points at which stimulation variably produced naming errors at least half of the time. A 50% error rate was chosen as the cut-off point because a lower error rate (given the small number of samples per site) could not unequivocally be distinguished from the non-stimulation control trial error rates. The control trial error rate for patient 1 was 12.8%, for patient 2 was 15.5% and for patient 3 was 14.1%. Sites which had stimulation error rates of less than 50% are indicated by an open circle containing an asterisk. Blank circles indicate sites at which stimulation never produced a naming error in that patient.



exposed cortex of each patient. For patient 1, 28 sites were selected, for patient 2, 23 sites were selected and for patient 3, 26 sites were selected. These sites are indicated in Fig. 1 by open and filled circles. The mean number of stimulation samples per site was 2.3 for patient 1, 2.2 for patient 2 and 3.1 for patient 3. No site was stimulated consecutively; stimulation was not applied during consecutive naming slides. Stimulation was applied to a site only if the immediately preceding object had been named correctly. Non-stimulation control trials were determined by the same criterion, the immediately preceding object had been correctly named.

We elected to score the errors conservatively, counting only omissions and substitutions, and disregarding both hesitations and slurring. If the correct name was spoken before the slide changed, it was counted as a correct trial; if the correct name was spoken after the slide changed, and therefore after stimulation had stopped, it was counted as an error. Substitutions, such as calling a 'fork' a 'spider', were counted as errors. Patient 1 made only one substitution error. Patient 2 made six substitution errors (one of which was the use of an English word for the object instead of the Dutch word). Patient 3 made 17 substitution errors, the only patient of the three who made a large number (43%) of this type of error. In all three patients, 197 stimulations were done at 77 sites, eliciting a total of 99 errors.

The results are depicted on the left hemisphere tracings for each patient in Fig. 1. These tracings were made directly from the venous phase of the angiograms; the sites were located in relation to the cortical veins, using colour photographs taken at the time of the language testing. The data provide an answer to the first question: in each case it was possible to find language functioning cortex throughout the extent of the left hemisphere exposure. Although this observation must be qualified by the fact that these are patients with long-standing epileptic foci, it seems reasonable to conclude that an individual's language system may occupy a very large extent of the lateral cortex, including the classical language zones derived from pooled data from many subjects. Indeed, our data show an even wider language zone than that proposed by Penfield and Roberts in that we find numerous language sites in the parietal operculum and mid-frontal and mid-parietal lobes. The answer to the second question seems to be no; the language function does not seem to be equally distributed throughout the left hemisphere. We repeatedly observed instances of a naming impairment on all stimulations at one site, and only partial or no impairments on all stimulations at an adjacent site, as represented in Fig. 1. The data suggest hypotheses of theoretical interest. If the patterns seen in these patients are indicative of language representation in non-epileptic left hemispheres, and there is no reason to assume that the non-epileptic cortex in these patients is abnormal, it is reasonable to infer that there is a marked degree of individual variation from one person to the next. If that inference is valid, it provides an obvious explanation for both the variety of aphasic symptoms and the variability of recovery of function which follow seemingly similar brain lesions.

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1. Penfield, W. & Roberts, L. *Speech and Brain Mechanisms* (Princeton University Press, Princeton, 1959).
2. Fedio, P. & Van Buren, J. M. *Brain Lang.* 1, 29-42 (1974).

## Infective *Theileria annulata* in the tick without a blood meal stimulus

THE agent of bovine tropical theileriosis, *Theileria annulata*, occurs in southern Europe, North Africa and most of Asia. The disease causes high mortality and reduced yield of meat and milk in the survivors. In enzootic areas theileriosis prevents the introduction of improved stock which are in most cases very susceptible. The work described here is part of a programme for improving the effectiveness of the vaccines now used for partial control of the disease. Theileriosis is transmitted in nature mainly by adult ticks that acquire their infection in the pre-imaginal stages. Adults become infective after feeding for a minimum of 2 d either on the susceptible bovine host or on a non-susceptible mammal like the rabbit<sup>1</sup>. As parasites can already be seen in the salivary glands of unfed adult ticks that are not infective<sup>2,3</sup>, it has been assumed that a blood meal provides the biological stimulus for the development of infectivity. In attempting to determine the factors other than feeding, that might stimulate the production of infective stage(s) of *T. annulata*, I have found that a temperature of 37 °C and a relative humidity (RH) of 95% is in itself sufficient to stimulate the production of infective parasite forms in infected adult *Hyalomma excavatum* ticks without the need for a blood meal.

Engorged nymphs of *H. excavatum* that were fed on a calf infected with *T. annulata* were maintained at 28 °C and 65% RH for 6 weeks and the adults that developed were kept for 3 months at 13 °C and 65% RH and served as the tick material for this study. When the adult ticks were allowed to feed for up to 2 d on susceptible calves, the animals were not infected, nor were calves which were injected with the homogenate of these ticks. Calves contracted theileriosis only after ticks had fed on them for 4-5 d, or after being injected with the homogenate of these ticks.

One hundred and thirty ticks from the same batch, enclosed in wire net containers covered with nylon net which prevented them from feeding, were placed in ear bags on a susceptible calf. After 2 d 65 of the ticks were transferred to the earbags on another susceptible calf for 24 h, during which time 38 ticks attached. The calf remained free of theileriosis. After 6 d the 53 confined ticks remaining on the initial calf were removed from the earbags, homogenised and injected into a fresh calf which died with fulminating theileriosis.

Unfed adult ticks were transferred from 13 °C and 65% RH to an incubator maintained at 37 °C and 95% RH for 6 d. The homogenate of 56 such ticks caused lethal theileriosis when injected into a susceptible calf. Unfed ticks which were kept at 13 °C, or ticks allowed to feed on calves for up to 2 d, did not transmit theileriosis either by bite or by needle inoculation.

From these results it seems that a temperature of 37 °C for several days, possibly coupled with high relative humidity, stimulated the non-infective particles of *T. annulata* in the salivary glands of the unfed *Hyalomma* ticks to become infective without the previous stimulation of feeding. These findings might explain the results of Mazlum<sup>4</sup> who was able to transmit *T. annulata* by injecting unfed infected adult *H. dromedarii* into cattle. Although Mazlum gave no explanation for his unexpected results, the fact that his ticks had been reared at 32 °C and 70-80% RH might have been a significant factor in the experiment.

The unsuccessful attempt by Nuttal and Hindle<sup>5</sup> to transmit *Theileria parva* by keeping unfed *Rhipicephalus appendiculatus* ticks at 37 °C for 3 d and feeding them on cattle for an additional 2 d, might be related to the species of ticks and the *Theileria* that they studied. The possibility that body temperature of the host, rather than tick feeding *per se* might be responsible for the appearance of infectivity in some tick-borne diseases is supported by the finding<sup>6</sup> that infective forms of *Babesia bovis* can be produced in unfed *Boophilus microplus* larvae by keeping them



at 37 °C. An elevation in temperature not only causes the appearance of infective particles, but also activates the ticks in general.

Failure to prevent theileriosis in hot climates by dipping cattle in acaricides every 3 d or even daily<sup>7</sup> might possibly be explained by the rapid appearance of infective particles in ticks exposed to high ambient temperatures, even before they have had a blood meal.

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Received 18 July; accepted 16 September 1977.

1. Samish, M. & Pipano, E. *Int. Conf. Tick-Borne Disease Vectors Univ. Edinburgh* (1976).
2. Cowdry, E. V. & Ham, A. W. *Parasitology* 24, 1-49 (1932).
3. Sergent, E., Donatien, A., Parrot, L. & Lestoquard, F. *Arch. Inst. Pasteur Alger* 14, 259-294 (1936).
4. Mazlum, Z. *Parasitology* 59, 597-600 (1969).
5. Nuttall, G. H. F. & Hindle, E. *Parasitology* 6, 321-332 (1913).
6. Daigleish, R. J. & Stewart, N. P. *Aust. vet. J.* 52, 543 (1976).
7. Barnett, S. F. *Theileriosis in Infectious Blood Diseases of Man and Animal* (eds Weinman, D. & Ristic, M.) 2, (Academic, London, 1968).

## Endosymbiont as supplier of ornithine carbamoyltransferase in a trypanosomatid

In spite of the widespread occurrence of intracellular bacteria-like symbionts in eukaryotic cells little is known about their function<sup>1-3</sup>. They are generally thought to offer some advantage to the host cell while benefiting from its 'hospitality', but their precise contribution is known in few cases. Flagellates that harbour endosymbionts provide excellent models for the analysis of this relationship. Three trypanosomatids, *Crithidia oncopelti*, *C. deanei* and *Blastocrithidia culicis* harbour endosymbionts whose bacteria-like nature has been confirmed morphologically and biochemically<sup>4-9</sup>. Unlike symbiont-free species<sup>10</sup>, those species do not require haemin for growth because the endosymbionts provide haemin-synthesising enzymes<sup>11</sup>. We now report that endosymbionts enable certain species of *Crithidia* to synthesise arginine from ornithine.

Although ornithine carbamoyltransferase (OCT) is characteristically absent from *Crithidia*, it is present in species harbouring symbionts (Table 1). OCT catalyses the first step in the synthesis of arginine from ornithine—the synthesis of citrulline from ornithine and carbamoyl phosphate. Because of its absence, endosymbiont-free *Crithidia* have an absolute growth requirement for arginine or

Table 2 Growth of three species of *Crithidia* in various media

Organism	<i>C. deanei</i>		<i>C. fasciculata</i>		<i>C. acanthocephali</i>	
Medium	GT	Cells per ml*	GT	Cells per ml	GT	Cells per ml
RDM	8	6 × 10 <sup>7</sup>	0	0	0	0
RDM+Orn	6.5	4 × 10 <sup>7</sup>	0	0	0	0
RDM+Cit	9	7 × 10 <sup>7</sup>	10	4 × 10 <sup>7</sup>	6.5	3.6 × 10 <sup>7</sup>
RDM+Arg	6.5	7 × 10 <sup>7</sup>	10	4.5 × 10 <sup>7</sup>	6.5	4 × 10 <sup>7</sup>

RDM, Roitman's defined medium<sup>17</sup> without arginine. RDM+Orn contains 3 mM L-ornithine; RDM+Cit contains 2.3 mM L-citrulline and RDM+Arg contains 2.3 mM L-arginine. GT is a rough calculation of the generation time in hours derived from inspection of growth curves.

\*Number of cells per ml, corresponding to measurements made at the stationary phase of the cultures, before cell decay.

citrulline. *Crithidia deanei* (Table 2) and *C. oncopelti*<sup>12</sup>, however, which have endosymbionts can grow in the absence of both amino acids, which indicates that they can synthesise citrulline through the mediation of OCT.

To investigate the role of the endosymbionts, we tested symbiont-free *C. deanei* and *C. oncopelti* for OCT (these strains are obtained by prolonged treatment with chloramphenicol (ref. 8 and personal communication from M. H. Mundin and I. Roitman)). Extracts of the strains showed no OCT activity (Table 1), suggesting that the symbionts provide OCT for *Crithidia*. But because chloramphenicol treatment is likely to affect the mitochondria<sup>13</sup>—reported to be the site of OCT in eukaryotes<sup>14-16</sup>—it is possible that the loss of OCT from *C. deanei* and *C. oncopelti* was due to impairment of protein synthesis in their mitochondria rather than to removal of symbionts.

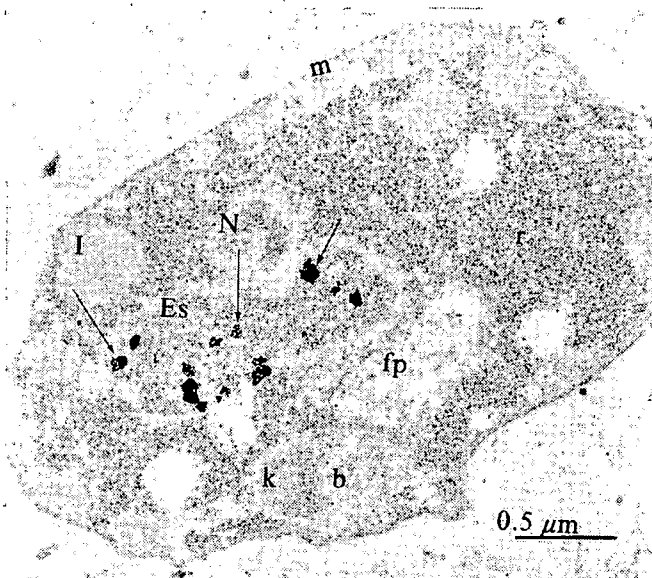
Our efforts to isolate the symbionts and locate OCT within them were inconclusive—we produced subcellular

**Fig. 1** Histochemical demonstration of OCT in *Crithidia deanei* which contains an endosymbiont. Arrows indicate OCT reaction product; Es, endosymbiont; N, nucleus; fp, flagellar pocket; k, kinetoplast; b, blepharoplast; l, lipid droplet; m, mitochondrion. Pellets of log-phase cells were fixed for 30 min at 4 °C in glutaraldehyde (2.5% in 0.1 M cacodylate buffer, pH 7.2). After several rinses, pellets were incubated with 5 mM lithium carbamoyl phosphate; 3 mM lead nitrate; 5 mM trisodium citrate; 50 mM Tris-HCl, pH 8.5, and 10 mM L-ornithine for 20 min at 37 °C. In control preparations ornithine was omitted from the incubation mixture. After rinsing with Tris buffer, pellets were included in 3% agar. Small agar blocks were post-fixed in osmium tetroxide (1% in cacodylate buffer, pH 7.2) for 1 h at 4 °C. After dehydration the material was embedded in Epon, sectioned, stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 101, 80 kV.

Table 1 Ornithine carbamoyltransferase in *Crithidia*

Organism	Enzyme specific activity
<i>C. fasciculata</i> (ATCC 11745)	0
<i>C. acanthocephali</i> (ATCC 30251)	0
<i>C. hamosa</i> (ATCC 30256)	0
<i>C. lucillae</i> (ATCC 14765)	0
<i>C. deanei</i> (ATCC 30255)	300
<i>C. deanei</i> without symbiont	0
<i>C. oncopelti</i> (ATCC 30264)	210
<i>C. oncopelti</i> without symbiont	0

Cells were grown in a undefined medium<sup>17</sup>. Cell homogenates were obtained by sonication. OCT was determined as before<sup>18</sup>. The assay mixture consisted of 40 mM Tris-HCl buffer, pH 8.5, 16.6 mM L-ornithine, 16.6 mM carbamoyl phosphate and 0.8 mg of enzyme protein in a final volume of 0.3 ml. After 15 min of incubation at 37 °C, reactions were stopped by addition of 5% trichloroacetic acid, and the citrulline formed was measured in the supernatant by the Archibald's method<sup>19</sup>. Results are expressed in nmol of citrulline per mg of protein per min. *Crithidia deanei* without symbiont was obtained from Drs I. Roitman and M. Mundin, University of Brasilia. *C. oncopelti* without symbiont was obtained from Dr K. P. Chang, Rockefeller University, through Dr Roitman.



fractions contaminated with mitochondrial fragments. The site of OCT was identified by electron microscopy. Log phase *C. deanei* and *C. oncopelti* were incubated with ornithine, carbamoyl phosphate and lead nitrate as before<sup>15</sup>. In this procedure the orthophosphate released during the OCT reaction is trapped *in situ* as a lead phosphate precipitate which can be visualised in electron micrographs. More than 40% of the cells examined showed a lead precipitate. When present, the precipitate was inside the symbiont and nowhere else in the cell (Fig. 1). No precipitate was found in preparations treated in the same way but without ornithine during incubation, nor in cells of the symbiont-free strains incubated with complete medium. Clearly OCT is localised in the endosymbiont and confers a nutritional advantage on its protozoan host.

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1. Buchner, P. *Endosymbiosis of Animals with Plant Microorganisms* (Wiley-Interscience, New York, 1965).
2. Trager, W. *Symbiosis* (Van Nostrand-Reinhold, New York, 1970).
3. Margulis, L. *Origin of Eukaryotic Cells* (Yale University Press, New Haven, 1970).
4. Newton, B. A. & Horne, R. W. *Expl Cell Res.* 13, 563-574 (1957).
5. Gill, J. W. & Vogel, H. J. *J. Protozool.* 10, 148-152 (1963).
6. Marmur, J., Cahoon, M. E., Shimura, Y. & Vogel, H. J. *Nature* 197, 1228-1229 (1963).
7. Gutteridge, W. E. & Macadan, R. F. *J. Protozool.* 18, 637-640 (1971).
8. Chang, K. P. *J. Protozool.* 21, 518-521 (1974).
9. Mundim, M. H., Roitman, I., Hermans, M. A. & Kitajima, E. W. *J. Protozool.* 21, 518-521 (1974).
10. Chang, K. P. & Trager, W. *Science* 183, 531-532 (1974).
11. Chang, K. P., Chang, C. S. & Sassa, S. *Proc. natn. Acad. Sci. U.S.A.* 72, 2979-2983 (1975).
12. Newton, B. A. *Nature* 177, 279-280 (1956).
13. Kroon, A. M. *Biochim. biophys. Acta* 72, 391 (1963); in *Inhibitors Tools in Cell Research* 159-166 (Springer, New York, 1969).
14. Mizutani, A. J. *Histochem. Cytochem.* 15, 603-604 (1967).
15. Bernhardt, S. A. & Davis, R. H. *Proc. natn. Acad. Sci. U.S.A.* 69, 1868-1872 (1972).
16. Weiss, R. L. & Davis, R. H. *J. biol. Chem.* 248, 5403-5408 (1973).
17. Roitman, C., Roitman, I. & Azevedo, H. P. *J. Protozool.* 19, 346-349 (1972).
18. Nakamura, M. & Jones, M. E. in *Methods in Enzymology* 17A, 286-294 (Academic, New York, 1970).
19. Archibald, R. M. *J. biol. Chem.* 156, 121-142 (1944).

## Calcium mobilisation during reproduction in snail *Helix aspersa*

THE land snail *Helix aspersa* lays up to 100 eggs at a time, each with 0.6 mg of calcium in the form of calcite crystals. There is insufficient calcium in any part of the reproductive tract to account for this.

We have investigated egg shell calcification in *H. aspersa* and report here a substantial increase in blood calcium during this process while blood sodium and pH remain constant. Rapid calcium mobilisation from non-reproductive areas to the egg shell-forming uterus during this period results in a 70% elevation of the blood calcium concentration, involving an increase in both the bound and unbound fractions.

Of the 65 families of land pulmonate snails comprising about 15,000 species, members of at least 36 families lay eggs with coverings containing calcium carbonate<sup>1</sup>. The calcified deposits may take the form of individual crystals of calcite dispersed through a jelly coat as in *Helix* or a hard shell as in *Achatina* and *Strophocheilus*<sup>2</sup>. The egg shell of the snail has four features in common with that of the bird: (1) similarity of mineralogy, ultrastructural appearance and even size in such snails as *Strophocheilus*<sup>3</sup>; (2) deposition of the shell—concurrently with a small

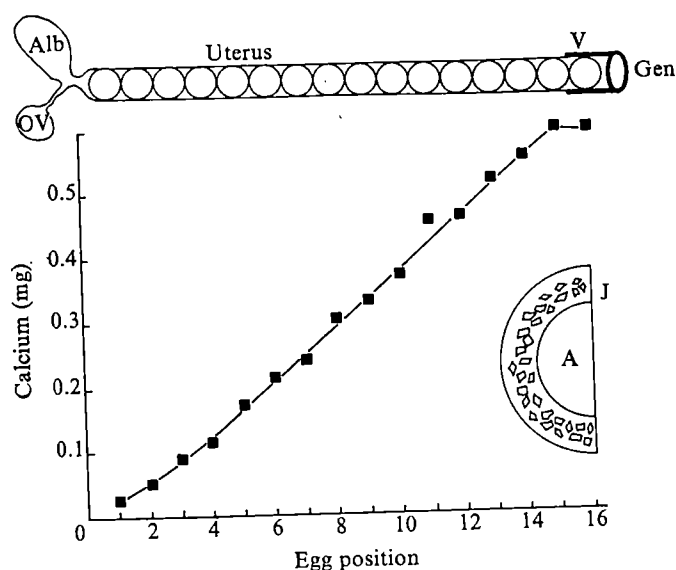


Fig. 1 Calcium content of the eggs as a function of location in the uterus of a gravid *Helix aspersa*. The eggs are in single file with the sides of the uterine wall closely applied, though each egg seems to be free to rotate. As soon as an egg is released anteriorly a new one is formed at the posterior end in an assembly-line fashion. The ovotestis (OV) and albumen gland (Alb) are shown not to scale, at the posterior end of the uterus. The uterine wall is ciliated and shows strong peristaltic waves moving anteriorly; the vagina (V) is highly muscular and aids in the final extrusion of the eggs through the genital opening (Gen). The calcium content of the uterus after all of the eggs were removed was 0.27 mg. Inset shows a cross-section through a fully calcified egg; the calcium of the egg is in the form of individual calcite crystals dispersed throughout the egg jelly (J), which surrounds the albumen fluid (A) in which the ovum floats. (Correlation coefficient for regression line,  $r = 0.99$ .)

amount of organic material for the shell matrix—during passage along a tube lined with epithelium; (3) a high rate of calcium transport across the epithelium during shell formation; and (4) utilisation of the shell during embryonic development, in the snail for the shell, in the bird for the bones<sup>4</sup>. On examining several species of pulmonate snails, we found no storage of calcium in the reproductive tract sufficient to account for calcification of even a small percentage of the eggs of a single clutch. Apparently in the snail—as in the bird—calcium stored in other parts of the animal is mobilised and then passes in the blood to the reproductive tract where it is transported across the epithelium to the egg. We have examined egg-shell calcification in the snail *Helix aspersa* and have measured accompanying changes in the blood.

Typically 25-100 eggs are deposited in a single laying period (depending on the size and health of the animal), with an egg released approximately every 15 min. Each deposited egg contains about 0.6 mg calcium requiring a mobilisation of up to 60 mg calcium. The calculated rate of calcium transport across the uterine epithelium of *Helix aspersa* is approximately  $3 \times 10^{-8} \text{ mol cm}^{-2} \text{ h}^{-1}$ , and in another land snail examined, *Anguispira alternata*, the rate is about  $1 \times 10^{-8} \text{ mol cm}^{-2} \text{ h}^{-1}$ . This compares with a calculated rate of about  $2 \times 10^{-8} \text{ mol cm}^{-2} \text{ h}^{-1}$  for the hen<sup>5</sup>.

Eggs taken from various positions in a gravid uterus of *Helix* were found to have increasing amounts of calcium as they passed along the uterus (Fig. 1), and the amounts were in direct proportion to the distance traversed. This result demonstrates that over most of its length the various regions of the uterus have the same capacity to deposit calcium during passage of the egg. One should note that the uterine epithelium is rather closely applied to the eggs and the eggs are uniformly distributed in single file and

barely separated. There is almost no free fluid in the uterine lumen which can be aspirated so that the eggs are definitely not floating. Free calcite crystals were not present, indicating that crystal nucleation was initiated by the jelly which coats the egg.

During egg laying a dramatic increase in total blood calcium of nearly 70% was found. The elevation was due to an equivalent increase in both the bound and unbound fractions. The blood sodium level did not change significantly and the pH remained at the normal level of about 7.80 (Table 1).

We measured blood calcium levels during the reproductive period as a function of the number of eggs already calcified, and samples were also taken before and after the period of egg laying. Blood calcium suddenly rose from 5.89 mM to 9.93 mM with the onset of egg laying and was maintained for the entire duration of laying (calcification) and then dropped back to the normal level within a few hours after the last egg was released (Fig. 2).

Blood changes in *Helix* differ from those which occur during egg laying in birds in three significant respects: (1) in *Helix*, both bound and ultrafilterable calcium increased, whereas in birds only bound calcium associated with the transport of yolk proteins increases, (2) in *Helix*, blood calcium elevation takes place within several hours of the time of release of the first egg, whereas in birds this occurs several days before egg formation, and (3) in *Helix*, the blood pH is unchanged whereas in birds it is decreased (for a general review of avian egg laying, see ref. 6).

Two aspects of particular interest relating to the rapid mobilisation and utilisation of a relatively large amount of calcium during reproduction in snails are the sources of calcium and the characteristics of calcium transport

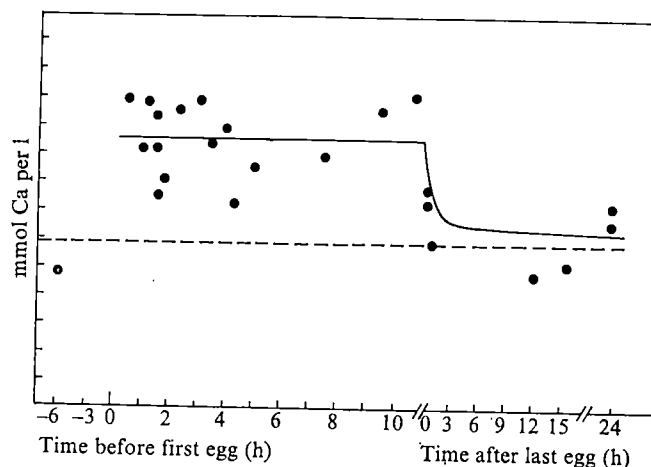


Fig. 2 Total calcium concentration of the blood of *Helix aspersa* at various stages of the egg-laying cycle. For each data point only a single sample of blood was taken from any given snail in order to avoid the lowering of calcium which otherwise may occur from trauma in laying animals (unpublished observations). Because of the great difficulty of predicting when an animal will initiate oviposition, only one point is available for the 6-h period before the appearance of the first egg. This sample represents blood calcium concentration only 2–3 h before egg shell calcification begins since it typically takes 3–4 h for a given egg to be fully calcified in passing through the entire length of the uterus. During egg shell calcification, represented on the graph by 0–11 h after the first egg laid, the blood calcium level was almost 70% higher than the normal level represented by the dotted line. This elevated blood calcium was maintained at a rather constant level as indicated by the regression equation for the line ( $y = 0.05x + 9.48$ ). Once the last egg was released, there was a sudden drop in the blood calcium level within 3 h and near normal values were reached a few hours later.

Table 1 Changes in blood of *Helix aspersa* during egg laying

	Normal	Egg-laying
*Calcium:		
total (mmol l <sup>-1</sup> )	5.89 ± 1.08 (14)	9.93 ± 1.29 (21)
% ultrafilterable	63 ± 3 (4)	61 ± 6 (4)
Sodium:		
total (mmol l <sup>-1</sup> )	65.7 ± 2.1 (9)	64.9 ± 3.4 (4)
pH	7.80 ± 0.03 (5)	7.79 ± 0.05 (6)

*Helix aspersa* snails were obtained from California and kept in the laboratory in standard light conditions in groups of 20, matched for approximate size. For all data on egg-laying snails, controls were animals from the same container in which the ovipositing snails were found, thus minimising variability in the degree of hydration, availability of food, and so on. All snails were kept in the active, non-aestivating state by providing high humidity, carrots, paper and calcium carbonate. Blood was taken by puncture of the vena pulmonalis immediately anterior to the auricle using a plastic syringe after the overlying shell area was quickly and gently removed. The pH of the blood was measured with a combination micro-glass electrode (MI Electrodes) on a Beckman expanded scale pH meter. Blood was drawn into a 1-ml plastic syringe; the hypodermic needle was immediately removed and the electrode inserted deeply into the syringe body containing the blood so that the tapered body of the electrode made an airtight fit with the syringe opening. Duplicate measurements were made within 45 s of sampling and no pH drift was observed over more than 5 min. (*Helix* blood freely exposed to air becomes more and more alkaline from loss of CO<sub>2</sub>.) For atomic absorption analysis of total blood calcium, a 10-μl sample of blood was diluted with 1.17% (w/v) La<sub>2</sub>O<sub>3</sub> in 5% (v/v) HCl (to prevent interference by elements such as aluminium, sulphate and phosphate) and then measured with a Perkin-Elmer 107 instrument. For sodium analysis, 10-μl samples of blood were diluted with distilled water and measured by flame emission spectroscopy. Blood was filtered at 15 pounds per square inch of N<sub>2</sub> or 2% CO<sub>2</sub>: 98% O<sub>2</sub> using a Clinical Ultrafiltration unit and an Amicon Diaflo Membrane of 1,000 MW cut-off. Ultrafiltration typically started with 1.0 ml of blood and no more than 50% of the fluid was allowed to pass through before the filtrate was collected. The amount of calcium in the filtrate is called unbound (mostly ionic and some complexed) and the remainder which is unfilterable is called bound. Values are mean ± standard deviation; sample size in parentheses.

\*P < 0.01.

across the uterine epithelium. These are being examined.

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1. Tompa, A. *J. Morph.* 150, 861–888 (1976).
2. Tompa, A. *Malac. Rev.* 7, 49–50 (1974).
3. Tompa, A. thesis, Univ. Michigan (1974).
4. Tompa, A. *Nature* 255, 232–233 (1975).
5. Romanoff, A. *The Avian Egg* (Wiley, New York, 1949).
6. Simkiss, K. *Calcium in Reproductive Physiology* (Reinhold, New York, 1967).

## Inhibition of post-implantation development of mouse blastocysts *in vitro* after cyclophosphamide treatment *in vivo*

It has been widely believed that embryos, after treatment in the preimplantation period, either die before implantation or survive to term without being malformed<sup>1,2</sup>. But evaluation of the embryolethal effect of cyclophosphamide (CPA) before implantation in the rat has revealed effects of the early treatment on both embryo and mother around and after implantation<sup>3</sup>. Further investigations of the development of CPA-treated preimplantation rat embryos are impossible because rat embryos have not been cultured successfully *in vitro* beyond the time of implantation. On the other hand, routine culture of mouse embryos *in vitro* during the same period has been standardised<sup>4–6</sup>. Such systems are sufficiently sensitive for investigations of genetically-determined abnormalities<sup>7,8</sup> and the effects of *in vitro* exposure to ultraviolet and X irradiation<sup>9,10</sup>.

**Table 1** Cell number of mouse embryos at implantation (day 3), 24 h after CPA treatment of the mother

Developmental stage	Dose (mg kg <sup>-1</sup> )	Cell no. per embryo	No. of determinations
Morula (control)	0	25.9 ± 4.8	18
Blastocyst (control)	0	42.3 ± 11.3	25
Blastocyst (treated)	20	34.5 ± 13.0	17
Blastocyst (treated)	40	21.2 ± 3.7*	35
Blastocyst (treated)	60	18.4 ± 4.6†	43
Blastocyst (treated)	80	15.9 ± 2.8†	63

Determinations were performed at 1400 h on day 3 as described by Tarkowski<sup>19</sup>. At this time the number of embryos per mother ( $7.0 \pm 2.7$  in 148 determinations), the ratio blastocysts per morula ( $5/2$  in 148 determinations) and the mitotic index ( $4\% =$  cells in mitosis expressed as percentage of total cells of the embryo) were unaffected by any of the CPA doses tested. The cell numbers of morulae from CPA-treated mothers were affected in the same way as were blastocysts. The maternal LD<sub>50</sub> for CPA was 200 mg kg<sup>-1</sup>.

\*Significantly lower than cell number of control blastocysts at  $P < 0.01$  (Wilcoxon-test).

†Significantly lower than cell number of both control blastocysts and morulae at  $P < 0.01$  (Wilcoxon test).

<sup>3</sup>H-thymidine<sup>11</sup> and metabolic inhibitors<sup>12-14</sup>. To elucidate further the action of CPA on the embryos after maternal treatment during the pre-implantation period, we have used mouse blastocysts to repeat our studies with CPA in the rat, and we have also cultured mouse blastocysts for 120 h from CPA-treated mothers. With concentrations of CPA that did not affect the morphology of blastocysts from treated mothers but only the cell number at the time of implantation, development of the blastocysts *in vitro* was inhibited in a dose-dependent manner. This system holds promise as a test for putative teratogens.

To determine the rate of lethality at term after CPA treatment 24 h before implantation, groups of 10 pregnant NMRI mice (Zentralinstitut für Tierzucht, Hannover) received a single subcutaneous injection of CPA at 20, 40, 60 or 80 mg kg<sup>-1</sup> (a gift from Asta-Werke AG) on day 2 (day 0 = the day after the mating night). At term the resorption rates for the four doses tested were 35, 78, 100 and 100%. As described for the rat<sup>3</sup>, CPA did not reduce the number of embryos per mother before and around the time of implantation but significantly decreased the number of living embryos during organogenesis. As with the rat, no malformations could be detected and the somite numbers of treated mouse embryos surviving to organogenesis were significantly lower than for controls, indicating a retardation of development of about 24 h. This could have been explained by a delay in implantation and so the exact time of implantation was determined as before<sup>3</sup>. Again as in the rat, there was no indication of delayed implantation in the treated group. But at CPA concentrations of 40–80 mg kg<sup>-1</sup> (Table 1) the cell numbers of treated blastocysts were significantly smaller than those of controls and of the 20 mg kg<sup>-1</sup> group ( $P < 0.01$ , Table 1). At the highest CPA concentrations (60 and 80 mg kg<sup>-1</sup>) the cell numbers of blasto-

cysts were significantly smaller than those of normal morulae (Table 1). The considerably smaller cell numbers in blastocysts of treated animals is evidence for interference by CPA or one of its metabolites with embryonic development *in vivo* during the 24 h between days 2 and 3. At the same time there was histological evidence of a simultaneous inhibition of the decidual reaction of the uterus, as in the rat<sup>3</sup>.

Blastocyst transplantation has been used to show the maternal CPA treatment has a direct effect on pre-implantation rat embryos<sup>3</sup>. But with the mouse a different approach was used. Blastocysts treated *in vivo* were cultured *in vitro* in conditions such that differentiation usually proceeds as follows<sup>5</sup>: hatching from the zona pellucida after 36–48 h; attachment to the surface of the dish after 48 h, and outgrowth of three characteristic cell types—a trophoblast layer with trophoblast giant cells and an inner cell mass (ICM) consisting of two germ layers, ectoderm and endoderm—which is usually completed after 96–120 h. In our culture system embryos from CPA-treated mothers showed a dose-dependent inhibition of all steps of differentiation (Table 2). The percentage of embryos which underwent ICM differentiation into two germ layers was the most sensitive parameter. It was already significantly decreased at the lowest CPA concentration (20 mg kg<sup>-1</sup>), which had no significant effect on the cell number of the blastocysts (Table 1). Studies of treatment *in vitro* have revealed a higher sensitivity of the ICM cells than of trophoblast cells to substances which interfere with nucleic acid metabolism<sup>11-14</sup>.

Other investigators have reported morphological abnormalities in pre-implantation rat<sup>16</sup> and mouse<sup>17</sup> embryos after treatment of the mother. The morphology of early rat<sup>3</sup> and mouse embryos was not affected by CPA as far as blastulation is concerned. But the cell number of CPA-treated blastocysts was considerably smaller than normal. This effect of CPA on *in vivo* development of pre-implantation embryos between the eight-cell (day 2) and the blastocyst stage (day 3) indicates that blastulation, the first step of morphological differentiation, is not dependent on the presence of a particular number of cells and that cells, having lost the capacity to divide at a normal rate, can still form the blastocyst cavity. The effects of CPA on subsequent development *in vitro* might be explained by a retarded clearance of the alkylating agent or one of its metabolites from the blastocyst cavity<sup>3,18</sup>.

For the evaluation of the effects of teratogens on pre-implantation embryos, the *in vitro* approach has several advantages over embryo transfer for which success has been low and poorly reproducible<sup>18</sup>. The *in vitro* system requires fewer embryos, and it is faster and more precise because maternal factors and individual variations are not involved. It also gives clearcut dose-response relationships, which are usually not obtained when treated embryos are transferred<sup>19</sup>.

Our technique analyses only the direct effect of the teratogen on the embryo itself and does not allow an assessment of the influence of a disturbed maternal physiology on later development. The detection of maternal effects of the early treatment

**Table 2** Effect of cyclophosphamide (CPA) treatment *in vivo* on differentiation of mouse blastocysts *in vitro*

CPA dose (mg kg <sup>-1</sup> )	No. of blastocysts (100%)	Hatching (%)	Attachment and outgrowth (%)	Extensive trophoblast growth (%)	ICM 2 germ layers (%)
0	98	97	97	96	93
20	119	89*	91	82†	61‡
40	73	81	80*	71*	25†
60	135	62†	62*	50†	11†
80	50	30*	28‡	22‡	0†

Blastocysts were flushed from the uteri at 1400 h on day 3 (24 h after maternal treatment) and cultured in groups of 10 in medium NCTC-109 (Difco) supplemented with 10% foetal calf serum (Gibco) in plastic culture dishes at 37 °C in a humidified 5% CO<sub>2</sub> in air atmosphere, as described by Sherman<sup>5</sup>. Significance levels were determined by the  $\chi^2$  test separately for each step of differentiation by comparing the growth rates (as percentage of blastocysts cultured) at every CPA dose with the growth rate at the next lower CPA dose.

\*  $P < 0.05$ .

†  $P < 0.01$ .

‡  $P < 0.001$ .

still requires transplantation experiments. In our studies with the rat we were able to show an inhibitory effect of early CPA treatment on the maternal environment by transplanting untreated embryos to treated and untreated pseudopregnant foster mothers<sup>3</sup>. Furthermore, transfer experiments are always necessary to check whether *in vitro* treatment during the preimplantation period is able to induce malformations at term<sup>18,20</sup>, especially because term malformations have been induced after transfer of preimplantation mouse embryos treated *in vitro* with the insecticide captan<sup>21</sup>.

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1. Austin, C. R. *Nature* 244, 333-334 (1973).
2. Wilson, J. G. *Environment and Birth Defects* (Academic, New York, 1973).
3. Spielmann, H., Eibs, H.-G. & Merker, H.-J. *J. Embryol. exp. Morph.* (in the press).
4. Spindle, A. I. & Pedersen, R. A. *J. exp. Zool.* 186, 305-318 (1973).
5. Sherman, M. I. *Differentiation* 3, 51-67 (1975).
6. McLaren, A. & Hensleigh, H. C. in *The Early Development of Mammals* (eds Balls, M. & Wild, A. E.) 45-60 (Cambridge University Press, 1975).
7. Pedersen, R. A. *J. exp. Zool.* 188, 307-319 (1974).
8. Wudl, L. R. & Sherman, M. I. *Cell* 9, 523-531 (1976).
9. Pedersen, R. A. & Cleaver, J. E. *Exp. Cell Res.* 95, 247-253 (1975).
10. Goldstein, L. S., Spindle, A. I. & Pedersen, R. A. *Radiat. Res.* 62, 276-287 (1975).
11. Ansell, J. D. & Snow, M. H. L. *J. Embryol. exp. Morph.* 33, 177-185 (1975).
12. Sherman, M. I. & Atienza, S. B. *J. Embryol. exp. Morph.* 34, 467-484 (1975).
13. Rowinski, J., Solter, D. & Koprowski, H. *J. exp. Zool.* 192, 133-142 (1975).
14. Glass, R. H., Spindle, A. I. & Pedersen, R. A. *J. Reprod. Fert.* 48, 443-445 (1976).
15. Tarkowski, A. K. *Cytogenetics* 5, 394-400 (1966).
16. Hurley, L. S. & Shrader, R. E. *Nature* 254, 427-429 (1975).
17. Jaquet, P., Leonard, A. & Gerber, G. B. *Toxicology* 6, 129-132 (1976).
18. Spielmann, H. in *Current Topics in Pathology* 62 (eds Gropp, A. & Benirschke, K.) 87-103 (Springer, Heidelberg, 1976).
19. Bell, P. S. & Glass, R. H. *Fertil. Steril.* 26, 449-454 (1975).
20. Spielmann, H., Eibs, H.-G., Nagel, D. & Gregg, C. T. *Life Sci.* 19, 633-640 (1976).
21. Staples, R. E. in *New Approaches to the Evaluation of Abnormal Embryonic Development* (eds Neubert, D. & Merker, H.-J.) 71-81 (Thieme, Stuttgart, 1975).

## Carbon dioxide reversibly abolishes ionic communication between cells of early amphibian embryo

Low electrical resistance intercellular junctions have been found in a wide variety of adult tissues, both *in vivo* and in tissue culture<sup>1</sup> and in early embryos<sup>2</sup>. Vertebrate and invertebrate adult intercellular junctions are permeable to small ions and a variety of other molecules, of molecular weights possibly up to 1,000, as shown by the movement of tracers such as fluorescein<sup>3</sup> and transfer of nucleotides ('metabolic cooperation')<sup>3</sup>. In adult systems, such intercellular exchange is correlated with the presence of gap junctions<sup>4</sup>. In embryos, some form of specific junction is necessary to account for the observed electrical coupling after early cleavage stages<sup>5</sup> and the presence of gap junctions has been reported<sup>6-8</sup>. Since the low resistance intercellular pathway has been implicated in the control of spatial and temporal organisation during development<sup>9</sup>, the permeability of the embryonic junction assumes some importance. There is evidence suggesting that embryonic junctions are less permeable than adult junctions<sup>10-13</sup>, which we have recently confirmed<sup>14</sup>. Our experiments<sup>14</sup> suggested that there is selectivity in the gap junctional membrane which led us to predict that the junctional permeability would be sensitive to changes in intracellular pH. We present here results which confirm this prediction.

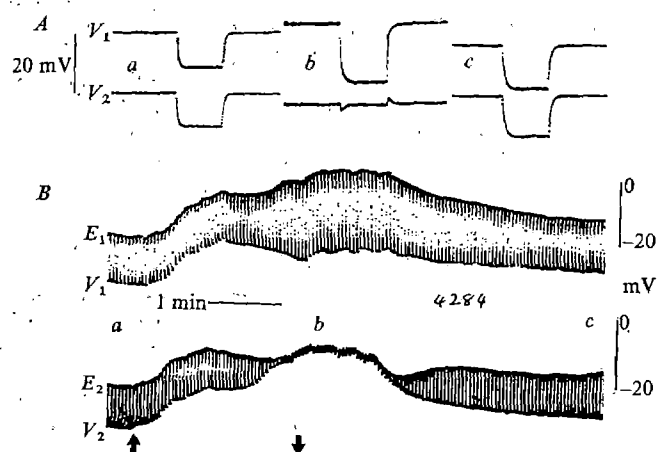
Electrical coupling between cells was measured in *Xenopus* embryos between the four-cell and mid-blastula stages. The embryos were continuously superfused with Holtfreter's solution (NaCl, 60 mM; KCl, 1 mM; CaCl<sub>2</sub>, 4 mM) buffered to

the required pH with 2 mM Tris hydroxymethylaminomethane or 10 mM ADA (*N*-(2-acetamido) iminodiacetic acid) (extracellular pH change) or 40 mM NaHCO<sub>3</sub>/CO<sub>2</sub> (intracellular pH change). Two glass intracellular microelectrodes (filled with 0.8 M potassium citrate) were inserted into one cell, one to record the membrane potential,  $E_1$ , and the voltage deflection,  $V_1$ , produced by a rectangular hyperpolarising current pulse ( $10^{-8}$  A, 1 s long, every 10 s) injected through the second microelectrode. A third microelectrode monitored the membrane potential in an adjacent cell,  $E_2$ , together with the voltage deflection produced by current flow through the intercellular junction,  $V_2$ . The ratio  $V_2/V_1$  gives a measure of the efficiency of electrical coupling between the cells. All parameters were displayed on an oscilloscope and slow writing chart recorder.

Extracellular pH changes between pH 5.8 and 7.5 had little effect on membrane potential, membrane resistance or electrical coupling. Intracellular pH was lowered by superfusing the embryo with a bicarbonate buffered Holtfreter solution saturated with 100% CO<sub>2</sub>; CO<sub>2</sub> crosses the membrane and forms carbonic acid which liberates H<sup>+</sup>, so lowering pH<sub>i</sub> (ref. 15). Figure 1 shows the effect of exposing a 64-cell embryo to 100% CO<sub>2</sub>; by contrast with an extracellular pH change, this brought about a rapid increase in junctional resistance, culminating in complete uncoupling of adjacent cells.

Table 1 summarises experiments on embryos between the 4-cell and blastula stages of development. In four- and eight-cell embryos lowering the intracellular pH had no effect on the coupling ratio  $V_2/V_1$ . Treatment with CO<sub>2</sub> produced a change in membrane potential, of variable magnitude and sign, at all stages of development tested, suggesting that depolarisation is not the cause of uncoupling; the membrane potential changes may be caused by an effect of CO<sub>2</sub> on the permeability of non-junctional membranes. When CO<sub>2</sub> uncoupled the cells there was invariably a concomitant rise in input resistance. Since CO<sub>2</sub> caused little change in resistance at the eight-cell stage it is unlikely that the uncoupling is brought about by the earthing of restricted extracellular current pathway normally responsible for current flow from one cell to the next. The most reasonable conclusion is that current flow from one cell to the next is largely mediated by a specialised intercellular pathway, whose permeability is greatly reduced when the embryo is

Fig. 1 Exposing a 64-cell embryo to 100% CO<sub>2</sub>. The pen record (B) begins with the embryo in Holtfreter solution at pH 7.5. The membrane potential was -17 mV and the coupling ratio, 0.9. Oscilloscope records of the individual electrotonic potentials  $V_1$  and  $V_2$  are shown above (A). At the arrow a Holtfreter solution buffered with 40 mM NaHCO<sub>3</sub> and equilibrated with 100% CO<sub>2</sub> (at pH 6.5) was admitted to the bath. The membrane potential in cell 2 decreased by 10 mV while the input resistance, recorded in cell 1, increased; at the same time electrical coupling between the cells disappeared. After 2.5 min uncoupling was complete, only capacitative artefact remains on the  $E_2$  trace. A (b), confirms the complete absence of an electrotonic potential in the adjacent cell. The return to Tris-buffered solution rapidly restored both membrane potential and electrical coupling (c).





**Table 1** Uncoupling induced by CO<sub>2</sub> at different developmental stages

	Initial resting potential (mV)	Potential during CO <sub>2</sub> (mV)	Final membranal potential (mV)	Effect of CO <sub>2</sub> on coupling
Stage 3 (4 cell)	20 11	6 0	8 0	None None
Stage 4 (8 cell)	11 11 10	5 6 10	5 8 13	None Partial uncoupling None
Stage 5 (16 cell)	26 16 18	10 3 5	18 15 18	Uncoupling Uncoupling Uncoupling
Stage 6 (32 cell)	21 10 8	0 0 0	24 8 10	Uncoupling Uncoupling Uncoupling
Stage 6½ (64 cell)	40 20 17 —	18 3 7 3	20 10 14 30	Uncoupling Uncoupling Uncoupling Uncoupling
Stage 7 (mid blastula)	— 20 25	3 5 5	33 25 30	Uncoupling Uncoupling Uncoupling

Membrane potential recorded at  $V_2$  electrode; all potentials negative with respect to zero. At 4- and 8-cell stage measurements made across first cleavage plane. Uncoupling means  $V_2/V_1$  falls to zero. No effect means normal  $V_2/V_1$  ratio (between 0.8 and 0.9).

exposed to CO<sub>2</sub>. The insensitivity of early cleavage stage embryos may reflect incomplete cleavage (the cells cleave every 20 min), or a change in membrane properties as the embryo enters the morula stage. In all other cases CO<sub>2</sub> completely abolished current flow from one cell to the next. This was always rapid and totally reversible.

Measurements of intracellular pH (using pH-sensitive micro-electrodes<sup>13</sup>) (in collaboration with R. C. Thomas) showed the pH to fall from a resting value of 7.7 to between 6.4 and 6.2 on exposure to 100% CO<sub>2</sub>. The intracellular pH change followed the same time course as the uncoupling. Extracellular pH changes between 5.8 and 7.5 had no effect on the intracellular pH.

The simplest interpretation of these results is that the effect of CO<sub>2</sub> treatment on electrical coupling between cells of the early *Xenopus* embryo is mediated by the low intracellular pH. An effect of anoxia is unlikely, since early amphibian embryos are notoriously resistant to oxygen lack<sup>14</sup> and we have found that treatment with 60% CO<sub>2</sub>: 40% O<sub>2</sub> is equally effective. It has been proposed that the permeability of the intercellular junction is controlled by the intracellular concentration of ionised calcium<sup>15</sup>. Since Meech and Thomas<sup>17</sup> have recently demonstrated that intracellular injection of Ca<sup>2+</sup> leads to a concomitant fall in intracellular pH, the possibility that a fall in intracellular pH rather than a rise in free Ca<sub>i</sub> was responsible for uncoupling observed in experiments such as those on the *Chironomus* salivary gland of Rose and Loewenstein<sup>18</sup> must now be considered. The question whether in our experiments the increase in intracellular H<sup>+</sup> displaces calcium from intracellular binding sites, so that intracellular calcium exerts the ultimate control remains open, although there are arguments against this possibility. We have injected iontophoretically into *Xenopus* embryo cells at the 64-cell stage sufficient calcium to bring about profound contracture of the contractile cytoplasm beneath the plasma membrane (making the free calcium level likely to be well above 10<sup>-8</sup> M (ref. 6)) without affecting the intercellular flow of current. 100% CO<sub>2</sub> did not produce any sign of contraction of the contractile cortex, despite its efficacy as an uncoupling agent, suggesting that no massive release of

calcium occurs. We therefore consider that a rise in Ca<sub>i</sub> was not primarily responsible for the uncoupling seen in *Xenopus* embryos on lowering intracellular pH.

The results described here open up a number of interesting possibilities. First it may be that the intracellular pH, rather than intracellular calcium, controls junctional permeability and similar experiments on the *Chironomus* salivary gland become important. Second, an increase in intracellular pH could cause a rise in junctional permeability and may provide a way of making the poorly permeable embryonic junction more like its adult counterpart. Finally, despite the difficulty of eliminating possible side effects of CO<sub>2</sub> treatment, our experiments offer, for the first time, the opportunity to test whether electrical coupling plays an essential role during development, as it should now be possible to reversibly uncouple the embryonic amphibian system and observe the developmental consequences. Experiments to answer these questions are already in progress.

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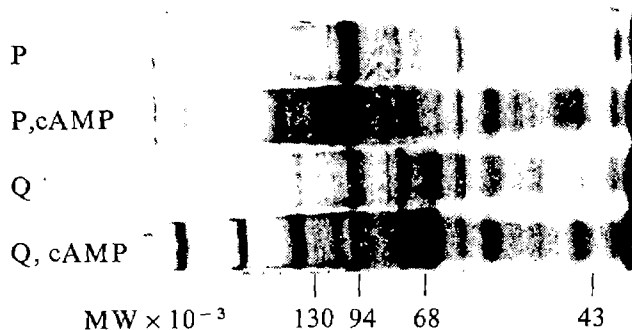
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1. Furshpan, E. S. & Potter, D. D. *Curr. Top. dev. Biol.* 3, 95-127 (1968).
2. Powers, R. D. & Tupper, J. T. in *Intracellular Communication* 231-251 (Plenum, New York, 1977).
3. Burk, R. R., Pitts, J. D. & Subak-Sharpe, J. H. *Expl. Cell Res.* 53, 297-301 (1968).
4. Gilula, N. B., Reeves, O. R. & Steinbach, A. *Nature* 235, 262-265 (1972).
5. Sheridan, J. D. in *Cell Surface Reviews* (eds G. Poste & G. L. Nicholson) 1, 409-443 (North Holland, Amsterdam, 1976).
6. Sanders, E. J. & DiCaprio, R. A. *Differentiation* 7, 13-21 (1976).
7. Sanders, E. J. & Zalik, S. E. *Wilhelm Roux' Arch.* 171, 181-194 (1972).
8. Ducibella, T., Albertini, D. F., Anderson, E. & Biggers, J. D. *Dev. Biol.* 45, 231-250 (1975).
9. Warner, Anne E. in *Simple Nervous System* 3-25 (Edward Arnold, London 1975).
10. Slack, C. & Palmer, J. F. *Expl. Cell Res.* 55, 416-419 (1969).
11. Tupper, J. T. & Saunders, J. W. *Dev. Biol.* 27, 546-554 (1972).
12. Bennett, M. V. L. in *Intracellular Staining in Neurobiology* 115-134 (Springer, Berlin, 1973).
13. Baker, P. F. & Warner, Anne E. *J. Cell Biol.* 53, 579-581 (1972).
14. Turin, L. J. *Physiol., Lond.* 269, 6P-7P (1977).
15. Thomas, R. C. *J. Physiol., Lond.* 238, 159-180 (1974).
16. Loewenstein, W. R. *Ann. N.Y. Acad. Sci.* 137, 441-472 (1966).
17. Meech, R. & Thomas, R. C. *J. Physiol., Lond.* (in the press).
18. Rose, B. & Loewenstein, W. R. *Nature* 245, 250-252 (1975).
19. Brachet, J. *Chemical Embryology* (Interscience, London, 1950).

## Unique cytoplasmic phosphoproteins are associated with cell growth arrest

THE intracellular molecular processes responsible for the regulation of animal cell growth are not understood. Cyclic AMP has been implicated in the arrest of cell growth (see ref. 1 for review) and it has been postulated that all cyclic AMP effects are mediated through protein kinase<sup>2</sup>. Insel *et al.*<sup>3</sup> have demonstrated with mutants that a cyclic AMP-dependent protein kinase mediates the growth inhibitory effect of cyclic AMP in S49 cells. Maller and Krebs<sup>4</sup> have established that the catalytic subunit of cyclic AMP-dependent protein kinase was both necessary and sufficient to block progesterone induced meiotic cell division in *Xenopus* oocytes. The above observations suggest that a high steady-state level of a phosphoprotein which is subject to control by cyclic AMP-dependent protein kinase functions in a regulatory manner to arrest cell growth. Since cyclic AMP-dependent protein kinases are found predominantly in the cytoplasm, we investigated the phosphorylation of cytoplasmic phosphoproteins and report here that quiescent cell cytosol contains several unique phosphoproteins.

The cell line used in this study was baby hamster kidney (BHK)<sub>21</sub> clone 13. Logarithmically growing cells were brought to quiescence by incubation in serum-deficient medium (0.1% calf serum in Dulbecco's modified Eagle's medium) for 40 h.



**Fig. 1** *In vitro*-labelled cytoplasmic phosphoproteins in quiescent and proliferating cells. Cytosol was prepared from quiescent (Q) and proliferating (P) BHK cells in the following manner. Cells on 100-mm dishes were washed twice with phosphate-buffered saline and scraped from the plate with a rubber policeman. The cells were centrifuged at 600g and the cell pellet washed twice more with phosphate-buffered saline. The cell pellet was resuspended in homogenisation buffer (10 mM HEPES (pH 7.4), 5 mM  $MgCl_2$ , 25 mM KCl), and cells were disrupted in a Potter-Elvehjem homogeniser. The homogenate was spun at 100,000g for 30 min and the supernatant (cytosol) dialysed for 12 h against the reaction buffer (100 mM NaCl, 20 mM HEPES (pH 7.2), 10 mM  $MgCl_2$ , 1 mM EGTA) at 4 °C. Inclusion of a protease inhibitor, phenylmethyl sulphonyl fluoride, in the dialysis buffer had no effect on the electrophoretic protein profile nor on phosphorylation. Cyclic AMP-dependent protein kinase activity was examined before and after dialysis using exogenous histone as substrate: no activity was lost in either cytosol preparation during dialysis. Cytosol protein concentration was adjusted to 2.5 mg protein per ml and theophylline (2 mM), NaF (10 mM), dithiothreitol (1 mM) and cyclic AMP (1  $\mu$ M, when present) were added. The reaction was initiated by the addition of  $\gamma$ - $^{32}P$ -ATP (2  $\mu$ M, 1,000–3,000 Ci mmol $^{-1}$ ) and was run for 5 min at 30 °C. The kinetics of phosphorylation revealed that maximum incorporation of  $^{32}P$  occurred by 5 min in both quiescent and proliferating cytosol proteins. The reaction was terminated by addition of electrophoresis sample buffer (1% SDS, 1% mercaptoethanol, 50 mM Tris pH 6.8, final concentrations), and boiling for 1 min. Proteins were separated by SDS-polyacrylamide slab gel electrophoresis<sup>5</sup>. Gels were stained with Coomassie blue in 45% methanol and 7.5% acetic acid, destained and dried. Phosphoproteins were visualised by exposure of the dried gel to X-ray film. Molecular weights of phosphoproteins were estimated by comparison with standard proteins ( $MW \times 10^{-3}$  shown below autoradiogram) as described by Weber and Osborn<sup>7</sup>. cAMP, cyclic AMP.

At this point over 95% of the cells were arrested in G<sub>1</sub> phase of the cell cycle as determined by flow microfluorimetry.

Cytoplasmic extract was prepared from quiescent and proliferating cells and subjected to *in vitro* phosphorylation with  $\gamma$ - $^{32}P$ -ATP. The cytosol proteins were then separated by sodium dodecylsulphate (SDS)-polyacrylamide slab gel electrophoresis, and the phosphoproteins were identified by exposing the dried gel to X-ray film. Approximately 20 phosphoproteins were identified in cytoplasmic extracts from both quiescent and proliferating cells in the absence of cyclic AMP; most of the phosphoproteins in quiescent and proliferating cytosol were identical and phosphorylated to the same extent (Fig. 1). But a protein of molecular weight (MW) 76,000 was consistently phosphorylated only in cytosol from quiescent cells. In proliferating cell cytosol a protein of MW 96,000 was more intensely phosphorylated than in quiescent cytosol (Table 1). The pattern of Coomassie blue stained proteins in quiescent and proliferating cells was identical.

Incubation of the cytosols with cyclic AMP resulted in several striking differences in the phosphoprotein profile. Five proteins were consistently phosphorylated only in quiescent cell cytosol in the presence of cyclic AMP (Figs 1 and 2). These proteins included two that were larger than 220,000 (largest band seemed to be a doublet), and proteins of MW 140,000, 76,000 and 66,000. Surprisingly, cyclic AMP always inhibited the phosphorylation of the 96,000 MW protein by 40% (Table 1). The presence of cyclic AMP stimulated the phosphorylation

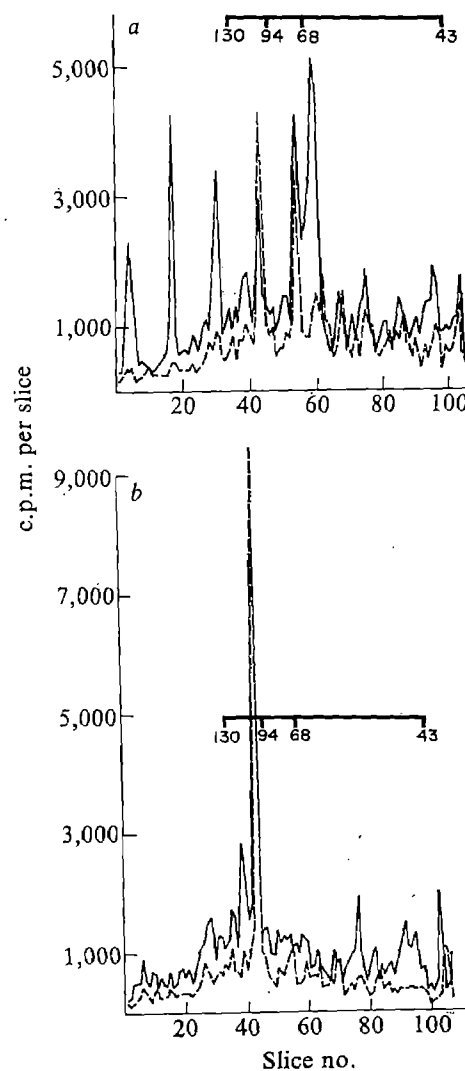
of only one protein (160,000 MW) unique to the proliferative growth state. All other cyclic AMP-stimulated phosphoproteins seemed to be in common with quiescent cell cytosol. Cyclic AMP also decreased phosphorylation of the 96,000 MW protein in proliferating cytosol (Table 1). Similar cyclic AMP effects were observed at concentrations of cyclic AMP from 0.1 to 10  $\mu$ M.

The same cyclic AMP-dependent phosphoproteins were observed when BHK cells were growth-arrested by isoleucine deprivation or serum deprivation, indicating that these phosphoproteins are associated with the quiescent state induced by two different conditions and are not simply the result of low serum in the culture medium.

The  $^{32}P$ -labelled bands in Fig. 1 were not destroyed by RNase, DNase, hot trichloroacetic acid or 1 M hydroxylamine. Protease treatment before electrophoresis destroyed all labelled bands as did 0.4 M NaOH at 37 °C for 18 h, indicating that the  $^{32}P$  bands represent phosphate covalently esterified to serine or threonine residues in cytosol proteins.

Wehner *et al.*<sup>6</sup> have reported that density-inhibited cells contain a 110,000 MW protein which is phosphorylated in the absence of exogenous cyclic AMP. We could not observe this

**Fig. 2** Quantitation of  $^{32}P$  incorporated into cytoplasmic phosphoproteins in quiescent and proliferating cells. The dried gel from the experiment depicted in Fig. 1 was cut into 2-mm slices and counted in a liquid scintillation counter. *a*,  $^{32}P$  incorporated into resting cell cytosol proteins in the presence (—) or absence (---) of cyclic AMP. *b*,  $^{32}P$  incorporated into proliferating cell cytosol proteins in the presence (—) or absence (---) of cyclic AMP.



**Table 1** Phosphorylation of the 96,000 MW protein in quiescent and proliferating cells and the effect of cyclic AMP

Source	<sup>32</sup> P c.p.m. per band*
Resting cell cytosol	8,490
Resting cell + cyclic AMP	5,022
Proliferating cell cytosol	27,689
Proliferating cell + cyclic AMP	21,920

Each cytosol was adjusted to 2.5 mg of protein per ml and subjected to phosphorylation conditions as described in Fig. 1. Exactly the same amount of protein was applied to the gel in each case. After the gel was dried and exposed to X-ray film, the 96,000 MW region was cut from the gel and counted in a liquid scintillation counter.

\*Average of two experiments

phosphoprotein in our studies: this may be because different cell lines were used in the two studies but important methodological differences may also explain this discrepancy. First, we used culture conditions in which the cells at the time of the experiment were at the same density but the growth state was modulated by the presence or absence of serum in the culture medium. Second, we dialysed the cytosol against the reaction solution before carrying out *in vitro* phosphorylation reaction. This brings the cytosol to specified pH and ion concentration and permits the study of cyclic AMP effects on phosphorylation. Wehner *et al.*<sup>6</sup> did not dialyse the cell extract and found no cyclic AMP effects that could be related to growth. Third, we used homogenisation in a Potter-Elvehjem homogeniser to disrupt cells and prepare cytosol while Wehner *et al.*<sup>6</sup> used sonication, a technique that damages membranes and disrupts cellular organelles, and carried out phosphorylation reactions on the total cellular sonicate. Thus, the relationship of the 110,000 MW phosphoprotein of Wehner *et al.*<sup>6</sup> to our data is not clear.

Results from several studies (reviewed in ref. 1) have established that elevated cyclic AMP is associated with the arrest of cell growth. The data presented here establish that quiescent cells consistently contain five unique phosphoproteins, four of which are phosphorylated only in the presence of cyclic AMP, and provide a possible mechanism by which cyclic AMP can control animal cell growth. The phosphoproteins may function as regulatory proteins to stop cell growth and maintain a G<sub>1</sub> block. Addition of mitogenic substances such as serum, which lower cyclic AMP<sup>8</sup>, to the culture medium would result in the dephosphorylation of the particular phosphoprotein(s) which maintains the G<sub>1</sub> block and thus, transit through G<sub>1</sub> is permitted. Other proteins may have to be phosphorylated or dephosphorylated at other points in the cell cycle to regulate progress through the cycle. An example of this would be the 96,000 MW protein which we have found to be intensely phosphorylated in proliferating cells by a cyclic AMP-independent protein kinase. The fact that cyclic AMP inhibits the phosphorylation of this protein is consistent with its possible role in promoting cell growth.

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- Pastan, I. H., Johnson, G. S. & Anderson, W. B. *A. Rev. Biochem.* **44**, 491-511 (1975).
- Kuo, J. F. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **64**, 1349-1353 (1969).
- Insel, P. A., Bourne, H. R., Coffino, P. & Tomkins, G. M. *Science* **190**, 896-898 (1975).
- Maller, J. L. & Krebs, E. G. *J. biol. Chem.* **252**, 1712-1717 (1977).
- Laemmli, U. K. *Nature* **227**, 680-682 (1972).
- Wehner, J. M., Sheppard, J. R. & Malkinson, A. M. *Nature* **266**, 842-844 (1977).
- Weber, K. & Osborn, M. *J. biol. Chem.* **244**, 4406-4412 (1969).
- Pledger, W. J., Thompson, W. J. & Strada, S. J. *Nature* **256**, 729-731 (1975).

## Tumour-dormant states established with L5178Y lymphoma cells in immunised syngeneic murine hosts

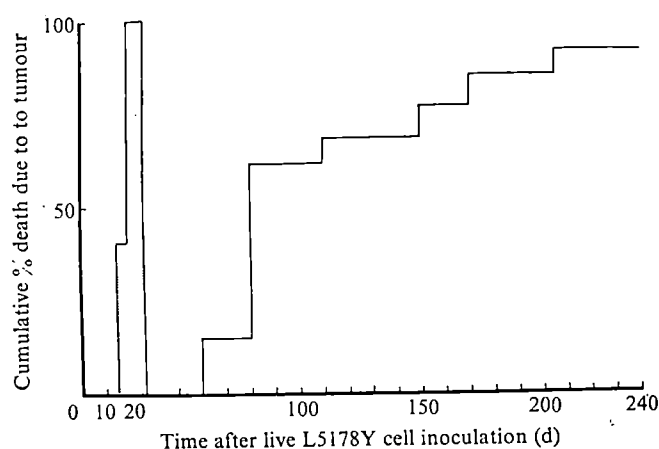
THE tumour-dormant state is one in which tumour cells persist in a clinically normal host for prolonged periods. Tumour dormancy is a potentially unstable state and may culminate in either destruction of all tumour cells or death of the host due to tumour outgrowth. We report here two systems in which tumour-dormant states could be established with transplantable lymphoma cells inoculated into specifically immunised syngeneic murine hosts. A high percentage of mice kept for long-term observation eventually developed tumours and in both systems, tumour cells could be isolated from mice during a prolonged period of clinical normality.

There are many reports describing the recurrence of solid tumours and leukaemias years after apparently successful treatment of the primary neoplasia<sup>1-3</sup>. These reports suggest that residual tumour cells can persist in a dormant state during periods of prolonged clinical remission, but do not rule out *de novo* recurrence of the same type of cancer due to a long lasting predisposition of the patient's cells to malignant transformation.

Since Fisher and Fisher's first report on tumour dormancy in rats<sup>4</sup>, only a few animal models have been developed for the study of this state<sup>5-8</sup>. Tumour dormancy in experimental animals may, however, be a frequent occurrence which goes unrecognised because experiments are usually terminated before tumour dormant cells emerge to produce overt tumours. We wish to make others aware that resistance of mice to tumour-cell challenge may be associated with prolonged tumour dormancy rather than tumour cell rejection.

In our experiments, we used the methylcholanthrene-induced lymphoma, L5178Y, an immunogenic, weakly metastatic tumour of DBA/2 origin<sup>9</sup>. In the first system, DBA/2 mice were immunised by intraperitoneal (i.p.) injection of 10<sup>7</sup> mitomycin C-treated L5178Y cells and thereby protected against the rapid outgrowth of live tumour cells inoculated i.p. 10 d later. Long-term observation of these mice revealed that 55-95% eventually developed tumours, some as late as 5 months after cell inoculation. In the experiment described here, immunised mice were killed on selected days after L5178Y cell challenge and attempts made to recover tumour cells from the peritoneal cavity and the spleen by *in vitro* culture. Additional mice from the same group were observed for extended periods for delayed tumour cell outgrowth. All non-immunised DBA/2 mice died of tumour growth within 25 d of i.p. inoculation of 5 × 10<sup>4</sup> live L5178Y cells, whereas no deaths occurred in the immunised, challenged mice until day 60, and by day 210, 93% of the mice had died of tumours (Fig. 1). Table 1 shows that tumour

**Fig. 1** DBA/2 females (8-10 weeks) were immunised by intraperitoneal (i.p.) injection of 10<sup>7</sup> mitomycin C-treated L5178Y cells. Control mice received an equivalent amount of PBS. All mice were given 5 × 10<sup>4</sup> L5178Y cells i.p. 10 d later. Shaded histogram, controls; open histogram, treated animals.



**Table 1** *In vitro* isolation of tumour cells from clinically normal mice previously immunised with mitomycin C-L5178Y cells

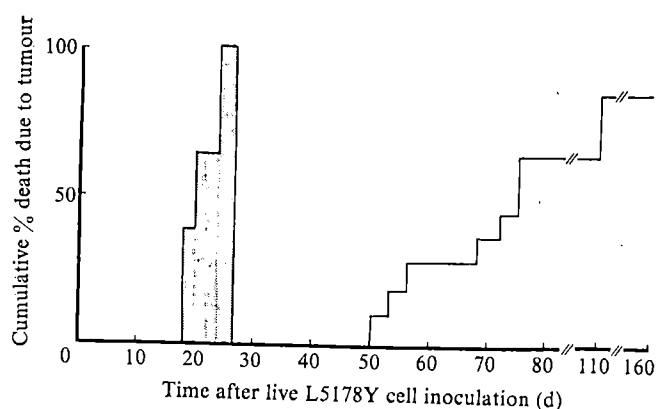
Time after tumour cell challenge (d)	Site of tumour cell isolation (No. mice with tumour cells/total no. mice tested)	
	Peritoneal cavity	Spleen
30	3/5	0/5
49	2/2	1/2
55	4/7	3/7
Total	9/14	4/14

Clinically normal mice (see text) were killed at the times shown. Their peritoneal cells and spleens were collected, washed and single cell suspensions of each were placed into culture at various concentrations and observed for 14 d. Cultures were judged positive for tumour cell outgrowth if increasing numbers of large lymphoblastoid cells appeared and continued to proliferate when transferred into new culture vessels.

cells could be isolated from cultures of peritoneal cavity cells and of spleen cells removed from these mice during the period when they were clinically normal. (Mice were judged to be clinically normal if total body weight did not exceed 30 g; the abdomen did not seem distended; macroscopic tumour foci were not visible following killing; and the total peritoneal cell count did not exceed  $1 \times 10^7$  cells.) The criterion for isolation of tumour cells *in vitro* was the appearance of increasing numbers of large lymphoblastoid cells which continued to proliferate following transfer into new culture vessels. These cells, when inoculated i.p. into normal DBA/2 mice, routinely produce ascitic tumours within 30 d, indicating that tumour cells recovered from dormant mice retain their tumorigenicity in normal syngeneic animals. An attempt was made to determine the period of persistence of tumour cells in a dormant state and we found that they could be recovered from the peritoneal cavity of clinically normal mice as long as 380 d after original tumour cell challenge.

To avoid the possible artefacts that occur when immunisation and tumour challenge are both given intraperitoneally, we used a sinecomitant immunisation procedure in our second system. DBA/2 mice were injected subcutaneously with  $1 \times 10^6$  L5178Y cells and 10 d later the small (1 cm) tumour nodules were excised. Such control mice remained free of tumour throughout a 160-d observation period, and no tumour cells could be isolated from their spleen or peritoneal cells, indicating that excision of the nodule had been complete and that no metastasis had occurred from the primary site of implantation. Excision of the tumour nodule protected mice against the rapid outgrowth of  $5 \times 10^4$  L5178Y cells inoculated i.p. 7 d later (Fig. 2); however, most of these challenged mice eventually developed tumours by day 160. Tumour cells could be isolated from the peritoneal cavity of many

**Fig. 2** DBA/2 females (8–10 weeks) were immunised by implantation of  $10^6$  live L5178Y cells subcutaneously on the ventral surface. Tumour nodules were removed surgically 10 d later, and mice were challenged 7 d later by i.p. inoculation of  $5 \times 10^4$  L5178Y cells. Control animals received PBS subcutaneously and  $5 \times 10^4$  L5178Y cells intraperitoneally at the appropriate times. Shaded histogram, controls; open histogram, immunised animals.



of the immunised and challenged mice while they were clinically normal (Table 2).

Results of experiments in both systems show that specific immunisation does not lead to complete elimination of all challenge tumour cells, but rather results in the establishment of a tumour-dormant state in a high percentage of animals. During this dormant state, tumour cells which were restrained from outgrowth *in vivo* grew out rapidly when transferred to *in vitro* culture. Although immunisation with mitomycin C-treated or X-irradiated tumour cells<sup>10,11</sup>, or with live tumour cells which were subsequently removed<sup>12,13</sup>, has previously been reported to protect mice against the rapid growth of a tumour cell challenge, in none of these studies were the mice observed long enough for dormant cells to emerge.

Essential to the tumour-dormant state is *in vivo* restraint of tumour cell outgrowth. In our systems a single surviving L5178Y cell doubling unrestrained at its normal *in vivo* rate ( $\sim 11$  h) can produce a pronounced ascites within 30 d—an interval which represents a small fraction of the observed tumour-dormant period of clinical normality. The possible mechanisms of tumour cell growth restraint *in vivo* include: continuous tumour cell division with concurrent tumour cell destruction; cytostatic effects which greatly prolong the *in vivo* population doubling time of the

**Table 2** *In vitro* isolation of tumour cells from clinically normal mice previously immunised by L5178Y tumour nodule formation and excision\*

Time after tumour cell challenge (d)	Site of tumour cell isolation (No. mice with tumour cells/total no. mice tested)	
	Peritoneal cavity	Spleen
40	0/2	0/2
50	0/2	0/2
55	0/2	0/2
60	3/3	0/2
65	1/1	0/2
72	3/3	1/3
75	1/2	1/2
Total	8/15	2/15

\*See legend to Table 1.

tumour cells; immunoselection of a tumour cell subpopulation which grows more slowly than the original population; and sequestration of residual tumour cells in anatomical sites where they can escape destruction but still be inhibited in their division rate. Eccles and Alexander<sup>5</sup> showed that tumour metastases may be maintained in a dormant state by immunological mechanisms, since immunosuppressive measures resulted in outgrowth of tumour cells.

The rapid *in vitro* outgrowth of tumour cells from peritoneal and spleen cell cultures prepared from tumour-dormant mice suggests that effector cells or soluble factors that suppress tumour cell proliferation *in vivo* may be either lost or inoperative after removal from the animal. A related observation may be the "escape from cytostasis" described by Remington and coworkers<sup>14</sup>. They found that activated macrophages could inhibit <sup>3</sup>H-thymidine uptake by tumour cells for only 24 h after *in vitro* challenge, with renewed uptake of labelled thymidine observed thereafter. A similar tumour escape from host cytostatic mechanisms may be responsible for *in vitro* tumour cell outgrowth in our models.

Absolute identification of the origin of tumour cells emerging after the dormant period is very difficult in our syngeneic systems. We have, however, observed that tumour cells which emerged in dormant mice 80 d after challenge grow out rapidly when inoculated into normal mice but fail to grow out when inoculated into L5178Y-immunised mice, indicating that the emerging cells express L5178Y tumour-associated transplantation antigens. Additionally, DBA/2 mice have an extremely low incidence of spontaneous lymphomas and although electron microscopy of

L5178Y cells reveals the presence of a C-type virus, no infectious virus could be demonstrated by either S+L- or XC assays, and homogenates of L5178Y cells were not tumorigenic (unpublished results).

The two murine systems reported here are now being analysed to elucidate the mechanisms involved in the establishment and maintenance of the tumour-dormant state, with a view to identifying those events which lead to breakdown of the tumour-dormant state and development of overt neoplasia.

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1. Hadfield, G. *Br. med. J.* **2**, 607-610 (1954).
2. Gordon-Taylor, G. *Br. med. J.* **2**, 455-462 (1959).
3. Boyd, W. *The Spontaneous Regression of Cancer* (Charles C. Thomas, Illinois, 1966).
4. Fisher, B. & Fisher, E. R. *Science* **130**, 918-919 (1959).
5. Eccles, S. A. & Alexander, P. *Nature* **257**, 52-53 (1975).
6. Noble, R. & Hoover, L. *Cancer Res.* **35**, 2935 (1975).
7. Gimbrone, M. A., Jr., Leepman, S. B., Cotraw, R. S. & Folkman, J. *J. exp. Med.* **136**, 261-276 (1972).
8. Wheelock, E. F., Caroline, N. L. & Moore, R. D. *J. Virol.* **4**, 1-6 (1969).
9. Parr, I. *Br. J. Cancer* **26**, 174-182 (1972).
10. Natale, N., Reiner, J. & Southam, C. M. *Cancer* **28**, 1118-1125 (1971).
11. Benjami, E. *et al. J. Immun.* **118**, 685-693 (1977).
12. Riggins, R. S. & Pilch, Y. H. *Cancer Res.* **24**, 1994-1996 (1964).
13. Barski, G. & Youn, J. K. *J. nat. Cancer Inst.* **43**, 111-121 (1969).
14. Krehenbuhl, J. L., Lambert, L. H., Jr. & Remington, J. S. *Cell. Immun.* **25**, 279-293 (1976).

## Post-infection genetic resistance to avian lymphoid leukaemia resides in B target cell

NEOPLASMS in which the target for transformation is a B-dependent lymphoid cell include Burkitt's lymphoma<sup>1</sup> and chronic lymphocytic leukaemia<sup>2</sup> of man, bovine leukaemia<sup>3</sup> and lymphoid leukaemia in chickens<sup>4-11</sup>. Inherited resistance to the development of tumours may involve at the first level, a resistance of all cells in the body to infection by tumour viruses or, at the second level, a failure of the tumour to develop or progress in virus-infected animals<sup>12-14</sup>. In mice, the *H-2*-associated resistance to leukaemia is at the second level and is probably an example of immune surveillance, in which the immune system eliminates or prevents the multiplication of nascent malignant cells<sup>14-16</sup>. Possible effector mechanisms for immune surveillance which have been described include cytotoxic cells<sup>17</sup>, antibody<sup>18</sup> or non-lymphoid cells<sup>19,20</sup>. An alternative to immune surveillance is that genetic resistance may be expressed directly by the target

cells, involving resistance either to viral infection<sup>21</sup> or to malignant transformation. We have used transfers of bursal cells from genetically resistant chickens into bursa-lymphocyte-depleted genetically susceptible chickens, and vice versa, to show that genetic resistance to lymphoid leukaemia resides in the bursal target cell, and that neither non-B cells nor cooperation between non-B cells and B cells has a role in this resistance.

The genetically resistant chicks were from East Lansing inbred line 6 subline 1 (R), and the genetically susceptible chicks from the F1 cross of East Lansing inbred lines 15 subline 1 and 7 subline 2 (S)<sup>22</sup>. These chicks are semi-allogeneic, having major histocompatibility genotypes *B<sup>2</sup>/B<sup>1</sup>* and *B<sup>2</sup>/B<sup>1</sup>*, respectively. Thus, it was necessary for bursal cell transfers to be made into recipients at 3 d of age, that is, within the tolerance-responsive period, in order to ensure successful bursal reconstitution in the histoincompatible transfers<sup>23</sup>. In other respects the transfers were as previously described for syngeneic cells<sup>7</sup>. Recipient chicks were treated with three doses of 4 mg cyclophosphamide (Cytosan, Mead Johnson) on days 0, 1, and 2 after hatching and received intravenous injections of bursal lymphoid cell suspensions from 10-d-old donors on day 3. The doses were  $1.15 \times 10^8$  cells per recipient from susceptible donors, but only  $7 \times 10^7$  cells per recipient from resistant donors, since these donor chicks from line 6<sub>1</sub> had exceptionally small bursas. Groups of untreated and cyclophosphamide-treated chicks served as non-transferred controls. Within 1 d of the transfers, all chicks were infected intravenously (i.v.) with  $>10^4$  tissue culture infective doses of avian leukaemia virus (RAV-1), and were vaccinated against Marek's disease by intramuscular inoculation with approximately 2,000 plaque-forming units of turkey herpesvirus vaccine<sup>7</sup>. Tests for humoral immune function were performed at 16 weeks by inoculating killed *Brucella abortus* and sheep erythrocytes i.v. and bleeding for serum 1 week later<sup>7</sup>. All birds dying during the experiment were necropsied and survivors were killed and examined for lymphoid leukaemia at 33 weeks of age, using both gross and histopathological study. Table 1 includes the pooled data from two replicate experiments performed 1 month apart in which the results were similar.

Regardless of recipient genotype, cases of lymphoid leukaemia occurred only in chickens receiving bursal cells from donors of susceptible genotype (Table 1, groups 1 and 2), and no cases resulted from transfer of bursal cells of resistant genotype (groups 3 and 4). The incidence of lymphoid leukaemia in the successfully reconstituted recipients of susceptible-type bursal cells (groups 1 and 2) was lower than in the untreated susceptible controls (group 8). Successful B-cell functional reconstitution with bursal cells was achieved in almost all the syngeneic transfers (groups 2 and

Table 1 Effect of bursal cell transfer on antibody response and lymphoid leukaemia development in cyclophosphamide-treated chicks

Group	Donors Phenotype	Recipients		No. tested	No. (%) responders*	Antibody and LL among responders		
		Phenotype	Treatment			SE Titre+	Ba Titre+	%LL†
1	S	R	CY	39	15 (38)	10.4	10.3	50
2	S	S	CY	32	32 (100)	11.5	11.3	44
3	R	S	CY	20	10 (50)	8.0	6.8	0
4	R	R	CY	22	21 (95)	10.9	8.8	0
5	None	R	CY	26	0	—	—	0
6	None	S	CY	29	0	—	2.6	0
7	None	R	None	26	26 (100)	11.6	11.2	4
8	None	S	None	18	18 (100)	11.9	10.4	100

Groups of genetically resistant (R) or susceptible (S) recipients were treated with cyclophosphamide (CY) on days 0-2 after hatching and received i.v. transfers of bursal cells 3 d after hatching from 10-d-old R or S donors. At 16 weeks, all birds were injected i.v. with *Brucella abortus* (Ba) and sheep erythrocytes (SE), and bled for serum 1 week later. Results of two replicate experiments were similar and are pooled.

\*Responders to both SE and Ba. In group 6, 12/29 responded to Ba with very low titres (1-5 log<sub>2</sub>).

†Mean log<sub>2</sub> titre of responders to each antigen.

‡LL, lymphoid leukaemia.



4), as shown by the incidence and titres of antibody responses. Reconstitution was less effective in the semi-allogeneic transfers, being 50% in the histocompatible group (R→S) and 38% in the converse histoincompatible group 1. Nevertheless, the results for lymphoid leukaemia incidence, based on successfully reconstituted chickens only, were clear-cut. Similar results were obtained in experiments in which susceptible chicks were reconstituted with syngeneic bursal cells<sup>7</sup>. It seems that the chance of bursal cells becoming transformed by leukaemia virus may be somewhat reduced in some reconstituted chicks, even though there are sufficient stem cells available to repopulate the antibody-forming system.

In confirmation of our previous findings<sup>7</sup>, the effect of cyclophosphamide treatment was to lower lymphoid leukaemia incidence from 100% to zero in susceptible chickens (groups 6 and 8), accompanied by a near-total suppression of humoral immune responses. The very low incidence of lymphoid leukaemia in resistant chickens (4%, group 7) was reduced to zero by cyclophosphamide treatment (group 5), with total loss of antibody responses.

From the clear-cut evidence that differences in lymphoid leukaemia incidence between groups of birds with successful restoration of the bursal system are associated with the genotypes of the donors rather than of the recipients, we conclude that resistance to lymphoid leukaemia of line 6<sub>1</sub> chickens is conferred on them by the bursal cells and not by other cellular elements of the immune system such as thymic or thymus-derived cells or non-lymphocytes. Although it is possible that this resistance may reside in B cell-mediated specific humoral immunity alone, as may occur in feline leukaemia<sup>18</sup>, it seems more likely that an intrinsic inability of the bursal target cell to become infected or transformed is the major factor.

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- Klein, G. in *Herpesviruses* (ed. Kaplan, A.) 521-555 (Academic, New York, 1973).
- Aisenberg, A. C., Bloch, K. J. & Long, J. C. *Am. J. Med.* 55, 184-191 (1973).
- Muscoplat, C. C. *et al. Am. J. vet. Res.* 35, 593-595 (1974).
- Peterson, R. D. A., Burmester, B. R., Fredrickson, T. N., Purchase, H. G., & Good, R. A. *J. natn. Cancer Inst.* 32, 1343-1354 (1964).
- Peterson, R. D. A., Purchase, H. G., Burmester, B. R., Cooper, M. D., & Good, R. A. *J. natn. Cancer Inst.* 36, 585-598 (1966).
- Burmester, B. R. *Poultry Sci.* 48, 401-408 (1969).
- Purchase, H. G. & Gilmour, D. G. *J. natn. Cancer Inst.* 55, 851-855 (1975).
- Purchase, H. G. & Cheville, N. F. *Avian Pathol.* 4, 239-245 (1975).
- Payne, L. N. & Rennie, M. T. *Vet. Rec.* 96, 454-456 (1975).
- Cooper, M. D., Purchase, H. G., Bockman, D. E. & Gathings, W. E. *J. Immun.* 113, 1210-1222 (1977).
- Cooper, M. D., Payne, L. N., Dent, P. B., Burmester, B. R. & Good, R. A. *J. natn. Cancer Inst.* 41, 373-389 (1968).
- Crittenden, L. B. *Avian Dis.* 19, 281-292 (1975).
- Pearson, G. R. & Davis, S. *Cancer Res.* 36, 688-691 (1976).
- Pincus, T. & Snyder, H. W. in *Viral Immunology and Immunopathology* (ed. Notkins, A. L.) 167-187 (Academic, New York, 1975).
- Kripke, M. L. & Borsos, T. *J. natn. Cancer Inst.* 52, 1393-1396 (1974).
- Prehn, R. T. *Adv. Cancer Res.* 23, 203-236 (1976).
- Herberman, R. B., Holden, H. T., Tina, C. C., Lavrin, D. L. & Kirchner, H. *Cancer Res.* 36, 615-621 (1976).
- Essex, M., Sliski, A., Hardy, Jr, W. D. & Cotter, S. M. *Cancer Res.* 36, 640-645 (1976).
- Cerottini, J. & Brunner, K. T. *Adv. Immun.* 18, 67-132 (1974).
- Nathan, C. F., Hill, V. M. & Terry, W. D. *Nature* 260, 147-148 (1976).
- Gazzalo, L., Moscovici, M. G., Moscovici, C. & Vogt, P. K. *Virology* 67, 553-565 (1975).
- Stone, H. A. *ARS Tech. Bull.* 1514, 22 (US Government Printing Office, Washington DC, 1975).
- Toivanen, P., Toivanen, A. & Vainio, O. *J. exp. Med.* 139, 1344-1349 (1974).

## Two mechanisms of migration inhibition factor induction by tumour antigens

EVIDENCE has accumulated which stresses the importance of lymphokines in cell-mediated immunity both *in vivo* and *in vitro*<sup>1</sup>. But, the processes leading to release of lymphokines by antigen-activated lymphocytes, the mode of action of these lymphokines and their exact role in cell-mediated immunity are still not well defined. It has, however, been recognised that *in vitro* activation of immune lymphocytes by soluble antigens is macrophage dependent and that this activation is under the control of gene products of the major histocompatibility complex (*H-2* in the mouse)<sup>2</sup>. Using a Moloney murine sarcoma virus (MSV)-induced tumour system, we have shown, in agreement with reports of some other systems<sup>3,4</sup>, that immune T lymphocytes required macrophages for migration inhibition factor (MIF) production and, in addition, only histocompatible macrophages could assist immune T lymphocytes for MIF release after stimulation with soluble tumour-associated antigens<sup>5</sup>. We report here that gene products of the *H-2* complex regulated the macrophage-immune lymphocyte interaction for MIF release when soluble tumour extracts were used as the source of antigen. In contrast, when intact tumour cells were used as the source of antigen, a macrophage requirement was not detectable. Moreover, the direct lymphocyte-tumour cell interaction for MIF release was not under *H-2* restriction, since allogeneic as well as syngeneic tumour cells could activate MIF release from immune lymphocytes. These findings suggest the existence of at least two different pathways for MIF production, reflecting perhaps the activation of two distinct subpopulations of T lymphocytes or the activation of T lymphocytes at two different stages of differentiation.

To assess MIF production we used an indirect Agarose droplet assay<sup>6</sup>. Immune spleen cells (ISC) were removed from C57BL/6N (B6) mice 12-14 d after inoculation of a regressor strain of MSV. ISC were depleted of macrophages (d-ISC) by treatment with carbonyl iron powder and magnet<sup>6</sup> or by passage over rayon adherence columns<sup>6</sup> or Sephadex G-10 columns<sup>7</sup>. To generate MIF in the presence of soluble tumour-associated antigens, B6 d-ISC were incubated with normal peritoneal exudate cells (PEC) induced by light mineral oil and treated with anti-Thy-1.2 antisera plus complement. Two sources of soluble tumour-associated antigens were used: a 3 M KCl extract from Rauscher virus-induced ascitic lymphoma (RBL-5) and a 3 M KCl extract from chemically induced ascitic lymphoma [EL4 (G-)]. RBL-5 cells carry the relevant antigens to which B6 ISC are sensitised, whereas EL4 (G-) do not<sup>8</sup>. Alternatively, MIF was produced from B6 d-ISC by incubation with tissue culture RBL-5 cells or cryopreserved RLV or MLV ascitic tumour cells treated with puromycin (20 µg per 10<sup>7</sup> cells). 2-Mercaptoethanol at the final concentration of 5 × 10<sup>-5</sup> M was added to the cultures since it was found to increase the levels of MIF production by d-ISC. Each experiment was performed in quadruplicate and repeated at least 5 or 6 times. In the culture conditions used, NSC plus RBL-5 tissue culture cells or RBL-5 cells alone were found negative for MIF production as shown in a previous report<sup>5</sup>.

Table 1 shows MIF production by ISC in response to 3 M KCl soluble tumour extracts of RBL-5 cells while the ISC, depleted of macrophages, failed to release detectable amounts of MIF when stimulated with soluble tumour antigen. The reaction was antigen-specific because no MIF production was observed with 3 M KCl soluble extracts of EL4 (G-). However, we were able to restore the ability of d-ISC to produce MIF by the addition of syngeneic PEC or PEC from B10 mice sharing the same *H-2* complex, but differing at many other genetic loci from B6 mice. In

contrast, PEC from B10.A and B10.D2 mice which are congenic to B10 mice and differ only at the *H*-2 locus were not able to restore MIF production by B6 d-ISC in response to the antigen. Allogeneic PEC from BALB/c mice were also unable to cooperate with d-ISC in MIF production. Similar results have been observed with BALB/c mice bearing tumours induced by a regressor strain of Moloney murine sarcoma virus (unpublished). In a previous study<sup>3</sup> we showed that the inability of allogeneic macrophages to cooperate in MIF production was not due to a malfunction of macrophages or to an inhibitory effect in allogeneic cell mixtures, so these results indicate that the ability of macrophages to assist d-ISC in the production of MIF in response to a soluble antigen is controlled by gene products mapping in the *H*-2 complex.

Since *in vivo* lymphocytes come in contact with tumour antigens in both soluble form and in insoluble form (as tumour cells), we felt it was important to examine MIF production from immune lymphocytes stimulated with intact tumour cells instead of soluble tumour-associated antigens and to compare the role of macrophages with the two forms of antigen. Table 2 shows that B6 ISC, depleted of macrophages by different techniques, were able to produce MIF when the antigen was on intact tissue culture tumour cells. Moreover, MIF was already detectable at 4 h after initiation of culture while using soluble tumour extracts. MIF production required 14–16 h in addition to the preincubation of the soluble antigen with macrophages. Since the RBL-5 cells used were from an established culture cell line devoid of macrophages and the percentage of macrophages after treatment with carbonyl iron powder plus magnet in association with rayon adherence column was about 0.1%, as evaluated by latex particle ingestion and esterase staining<sup>10</sup>, MIF production in response to intact tumour cells as antigen did not have a detectable requirement for macrophages. As *H*-2 compatibility is required for the interaction between PEC and d-ISC in the induction of MIF by soluble antigen, it was of interest to determine if the same restriction held for the interaction between d-ISC and tumour cells in the generation of MIF. Table 3

**Table 1** Requirement of *H*-2 compatible macrophages for MIF production by B6 ISC in presence of soluble tumour-associated antigens

	Source of PEC	PEC <i>H</i> -2 type	3 M KCl RBL-5	3 M KCl EL4 (G-)
ISC	—	—	0.78*	0.98
d-ISC	—	—	1.07	1.05
"	B6	b	0.73	
"	B10	b	0.71	
"	B10.A	a	1.01	
"	B10.D2	d	1.07	
"	BALB/c	d	1.05	

B6 ISC ( $5 \times 10^6$  ml), untreated or treated by rayon adherence columns (d-ISC) and mixed with  $2.5 \times 10^5$  peritoneal exudate cells (PECs) from normal B6, B10, B10.A, B10.D2 and BALB/c mice, were incubated with 3 M KCl extracts of RBL-5 or EL4 (G-) tumour cells. After 4 h at 37 °C the cell mixtures were washed and resuspended in 1 ml of fresh medium containing 0.5% foetal bovine serum (FBS) for 42 h at 37 °C in 5% CO<sub>2</sub> atmosphere. The supernatants, adjusted to 10% FBS, were tested on normal B6 PEC induced by light mineral oil. Two  $\mu$ l droplets of Agarose medium solution, containing  $8 \times 10^5$  PEC, were placed with a microdispenser (Drummond Scientific) in the centre of each well of Falcon plastic Microtest II (3040) culture plates. Each droplet was allowed to solidify at room temperature for 5 min and then 0.1 ml of stimulated or control culture supernatants was added. After 24 h at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere the migration area images were projected, traced and measured by planimetry.

\* Migration index (M.I.) where

$$\text{M.I.} = \frac{\text{Migration area with stimulated supernatant}}{\text{Migration area with unstimulated control supernatant}}$$

All the M.I. under 0.85 were considered positive as determined previously<sup>3</sup>.

**Table 2** MIF production by macrophage-depleted B6 ISC in response to intact tumour cells

Treatment of ISC	Tumour Cells	
	RBL-5	EL4 (G-)
Carbonyl iron-magnet	0.37*	1.11
Rayon adherence column	0.64	0.97
Sephadex G-10 column	0.41	1.05
Carbonyl iron-magnet + rayon adherence column	0.52	1.02

B6 ISC ( $5 \times 10^6$ ), depleted of macrophages by various techniques, were mixed with  $5 \times 10^6$  RBL-5 tissue culture cells or EL4 (G-) tumour cells in 1 ml of medium containing 0.5% FBS and incubated for 48 h at 37 °C in 5% CO<sub>2</sub> atmosphere. The supernatants, adjusted to 10% FBS, were treated as described in Table 1.

\* Migration index calculated as in Table 1.

shows that d-ISC were able to produce MIF in response to tumour-associated antigens on allogeneic cells as well as on syngeneic tumour cells. At the ratio of 10:1 effector cells: tumour cell no activity was observed with normal spleen cells in the presence of allogeneic target cells, excluding the possibility of MIF release by allogeneic stimulation as has been observed with longer periods of incubation<sup>11</sup>.

These results demonstrated that tumour-associated antigens stimulate MIF production by immune lymphocytes by two different mechanisms. In the first one, involving soluble tumour-associated antigens, responses occur only when the antigens are presented on macrophages sharing the same *H*-2 of the mice used for *in vivo* sensitisation. In the second one, involving intact target cells, the tumour-associated antigens on the membrane of the tumour cells seem to be sufficient by themselves, independent of *H*-2 products on their surface and of macrophages, to stimulate the immune lymphocytes to produce MIF. The lack of *H*-2 restriction in the mechanism of direct lymphocyte-tumour cell interaction seen in this study is in agreement with other findings from our laboratory indicating that the killing of tumour cells by immune T lymphocytes as measured by 16–18 h <sup>51</sup>Cr assay is not under *H*-2 restriction<sup>12,13</sup>. Plata *et al.* also obtained results showing that *H*-2 restriction in the MSV system is not absolute<sup>14</sup>. The characteristics of the MIF response to soluble tumour antigens are similar to those observed in the induction phase of the immune response in other systems<sup>2</sup>, in which lymphocytes recognise soluble antigens only when bound to the *H*-2 products of the macrophage membrane, and our results are in accord with the 'complex antigenic determinant model' proposed by Thomas and Shevach<sup>15</sup>. On the other hand, the tumour-associated antigens presented on intact tumour cells would seem to allow stimulation of immune lymphocytes directly, without the requirement for macrophages or for similarity at the *H*-2 complex. This might be accounted for by a

**Table 3** Ability of macrophage-depleted B6 ISC to produce MIF in presence of syngeneic as well as allogeneic tumour cells

Tumour cells	<i>H</i> -2 type	Inducing agent	ISC	NSC
RBL-5	b	RLV*	0.76†	0.99
MBL2	b	MLV	0.73	1.07
EL4 (G-)	b	Benzpyrene	0.97	0.95
LSTRA	d	MLV	0.70	0.98
YC8	d	MLV	0.77	1.08
YAC	a	MLV	0.79	1.10

B6 ISC or NSC ( $5 \times 10^6$ ), depleted of macrophages by rayon adherence columns, were mixed with  $5 \times 10^5$  frozen tumour cells in 1 ml of medium containing 0.5% FBS and 2-mercaptoethanol ( $5 \times 10^{-5}$  M) for 48 h at 37 °C in 5% CO<sub>2</sub> atmosphere. The supernatants, reconstituted with 10% FBS, were tested as in Table 1.

\* RLV, Rauscher leukaemia virus. MLV, Moloney leukaemia virus.

† Migration index calculated as in Table 1.

reaction to cell-bound antigens by a different subpopulation of lymphocytes or by lymphocytes at a different stage of differentiation, perhaps the same subpopulation of cells which are capable of cytotoxicity against intact syngeneic or allogeneic tumour target cells (which also have no macrophage requirement). Alternatively, MIF production by immune lymphocytes to soluble tumour antigens versus intact tumour cells may be in response to different antigenic specificities, only one of which requires the aid of *H-2* compatible macrophages. In any event, the lack of *H-2* restriction in direct lymphocyte-tumour cell interaction has interesting implications for the mechanisms involved with *in vivo* tumour rejection, as it has been shown previously that by immunising with allogeneic BALB/c lymphoma (LSTRA), B6 mice could be protected from challenge with a syngeneic tumour (MBL-2)<sup>16</sup>.

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- David J. & David, R. *Prog. Allergy* 16, 300 (1972).
- Katz, D. H. & Benacerraf, B. *The Role of Products of the Histocompatibility Gene Complex in Immune Responses* (Academic, New York, 1976).
- Nelson, R. D. & Len, R. W. *J. Immun.* 114, 606-609 (1975).
- Wahl, S. M., Wilton, J. M., Rosenstreich, D. L. & Oppenheim, J. G. *J. Immun.* 114, 1296-1301 (1975).
- Landolfo, S., Herberman, R. B. & Holden, H. T. *J. Immun.* 118, 1244-1248 (1977).
- Kirchner, H., Chused, T. M., Herberman, R. B., Holden, H. T. & Lavrin, D. H. *J. exp. Med.* 139, 1473-1487 (1974).
- Ly, I. A. & Mishell, R. I. *J. Immun. Meth.* 5, 239-247 (1974).
- Herberman, R. B. *et al. J. natn. Cancer Inst.* 53, 1103-1111 (1974).
- Landolfo, S., Herberman, R. B. & Holden, H. T. *J. natn. Cancer Inst.* (in the press).
- Yam, L. T., Li, C. Y. & Crosby, W. H. *Am. J. clin. Path.* 55, 283-290 (1971).
- Gorski, A. J., DuPont, B., Hansen, J. A. & Good, R. A. *J. Immun.* 117, 865-870 (1976).
- Holden, H. T., Landolfo, S. & Herberman, R. B. *Transplant. Proc.* 9, 1149 (1977).
- Holden, H. T. & Herberman, R. B. *Nature* 268, 250-252 (1977).
- Plata, F., Jongeneel, V., Cerottini, J.-C. & Brunner, K. T. *Eur. J. Immun.* 6, 823-829 (1976).
- Thomas, W. D. & Shevach, E. M. *J. exp. Med.* 114, 1263-1275 (1976).
- McCoy, J. L., Fefer, A. & Glynn, J. P. *Cancer Res.* 27, 1743-1748 (1968).

## Correction of human mucopolidosis II enzyme abnormalities in somatic cell hybrids

THE mouse genome has been used to correct the abnormal enzyme phenotype of a human neurodegenerative disorder and to provide information on the nature of the molecular defect. This has been demonstrated in man-mouse somatic cell hybrids using human cells derived from an individual with mucopolidosis II. Mucopolidosis II (ML II) or I-cell disease is a rare fatal childhood storage disease which is associated with deficiencies and electrophoretic abnormalities of several lysosomal hydrolases<sup>1-4</sup>. Somatic cell hybridisation provides a methodology for examining the expression of mutant genes and for identifying and mapping genes associated with human enzyme abnormalities. We have examined the isozyme patterns of six enzymes altered in this disorder in man-mouse hybrids formed with fibroblasts from an ML II patient. These altered enzymes showed corrected patterns of expression in cell hybrids. Since several human enzymes are corrected, a defect in the post-translational processing of lysosomal enzymes is suggested. Previous studies fusing human lysosomal storage disease cells with rodent cell lines have failed to demonstrate correction of the enzyme deficiencies by the rodent genome<sup>5-8</sup>. We present here the first example of the correction of an abnormal enzyme

phenotype associated with a human lysosomal storage disease by fusing human and mouse genomes.

Children affected with the autosomal recessive ML II disorder show Hurler-like clinical features with growth and mental retardation and mucopolysaccharide and glycolipid storage culminating in death by age 2-6 yr (refs 1, 2). Cultured fibroblasts from ML II children show large numbers of cytoplasmic inclusions and deficiencies (2-10% of normal) of the lysosomal hydrolases  $\beta$ -hexosaminidase,  $\beta$ -glucuronidase,  $\beta$ -galactosidase,  $\alpha$ -galactosidase,  $\alpha$ -mannosidase, arylsulphatase, and  $\alpha$ -fucosidase<sup>3,4</sup>. Many of these enzymes are found at elevated levels in culture media and in serum<sup>4,9</sup>. Altered electrophoretic phenotypes have been observed for  $\beta$ -hexosaminidase<sup>10</sup> and several hydrolases excreted from ML II cells<sup>11</sup>. We have reported altered phenotypes for  $\beta$ -hexosaminidase, acid phosphatase<sub>2</sub>,  $\alpha$ -mannosidase, esterase-A<sub>4</sub>, and adenosine deaminase-d in cultured ML II fibroblasts<sup>12</sup>. These abnormalities provide markers for determining if the defect can be corrected by the addition of normal genetic material through cell hybridisation.

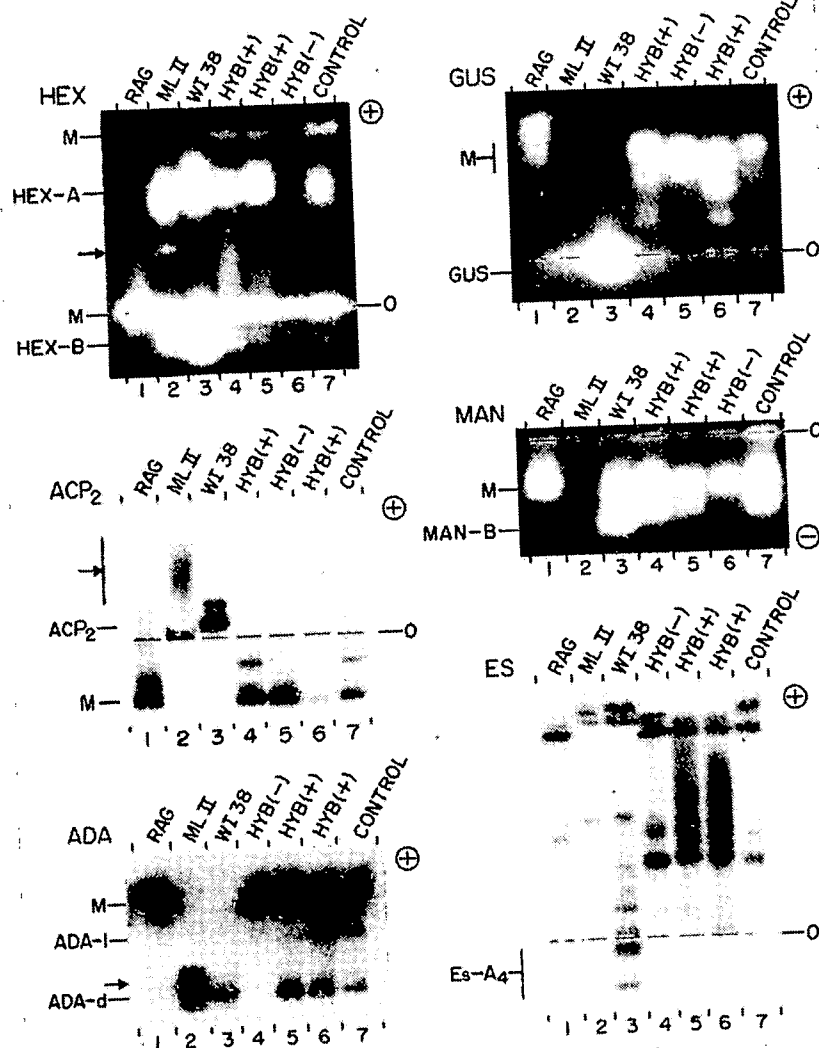
Mucopolidosis II fibroblasts (GM 1006, Human Genetic Mutant Cell Repository) were established from a female patient (L.T.) with ML II symptoms and multiple lysosomal enzyme deficiencies<sup>12,13</sup>. ML II fibroblasts were hybridised with two mouse cell lines: RAG (HPRT<sup>-</sup>) and LM/TK<sup>-</sup>, using Sendai virus as described previously<sup>14,15</sup>. Thirty-six independent clones were isolated consisting of 20 ML II  $\times$  RAG hybrids (ICR) and 16 ML II  $\times$  LM/TK<sup>-</sup> hybrids (ICL). Hybrid clones appeared with a frequency of about 10<sup>-4</sup>. Primary hybrids were selected, cloned, expanded and collected as described previously<sup>14</sup>. Enzyme assays of hybrids were accomplished at early passages<sup>3,4</sup> to maximise the number of human chromosomes retained in hybrids.

Enzymes with altered electrophoretic patterns in ML II fibroblasts were examined after cell hybridisation. Previously it was reported that  $\beta$ -hexosaminidase (HEX), acid phosphatase<sub>2</sub> (ACP<sub>2</sub>), and adenosine deaminase-d (ADA-d) have altered electrophoretic banding patterns in ML II fibroblasts<sup>10,12</sup>. The normal and ML II electrophoretic patterns of HEX, ACP<sub>2</sub>, and ADA-d in human fibroblasts are compared in Fig. 1. Mobility of these enzymes in ML II  $\times$  mouse cell hybrids was examined and compared with control hybrid patterns. Figure 1 shows that in ML II hybrids HEX, ACP<sub>2</sub>, and ADA-d were identical to controls. Thus,  $\beta$ -hexosaminidase showed normal mobility of the human HEX<sub>A</sub> and HEX<sub>B</sub> bands and failed to demonstrate the altered HEX band seen in ML II fibroblasts. Human ACP<sub>2</sub> was identified in hybrids predominantly as the man-mouse ACP<sub>2</sub> intermediate heterodimer and revealed normal electrophoretic mobility and expression. Human ADA-d was also identical to the ADA-d band in control hybrids.

Enzymes severely deficient in ML II fibroblasts were also examined in cell hybrids. Figure 1 demonstrates that the lysosomal enzymes  $\beta$ -glucuronidase ( $\beta$ GUS),  $\alpha$ -mannosidase (MAN<sub>B</sub>), and esterase-A<sub>4</sub> (ESA<sub>4</sub>) from extracts of ML II fibroblasts are weakly detected on starch gels. But human  $\beta$ GUS, MAN<sub>B</sub>, and ESA<sub>4</sub> in ML II  $\times$  mouse cell hybrids showed levels of expression on starch gels similar to control hybrids (Fig. 1). Human chromosomes are eliminated in man-mouse cell hybrids, so not every hybrid clone retained all the lysosomal enzyme markers. But, in each of the 36 hybrid clones examined, at least one of the lysosomal enzymes was retained and demonstrated the normal phenotype. A similar correction pattern was not observed by co-cultivation of mouse and ML II cells or by mixing of cell homogenates. The mouse enzyme pattern was never altered in hybrid clones.

It is possible that the chromosome on which the ML II gene is located is lost in these cell hybrids. Loss of the ML II chromosome might result in the corrected expression of the affected enzymes. Evidence supports complementation of the ML II defect since in 36 independent hybrids normal phenotypes of the enzymes were observed in each hybrid clone. The human chromosome complement in hybrid clones was determined

**Fig. 1** Starch gel electrophoretic patterns of lysosomal enzymes in ML II  $\times$  mouse cell hybrids. Enzymes shown include *N*-acetyl- $\beta$ -hexosaminidase (HEX); acid phosphatase, (ACP<sub>2</sub>); adenosine deaminase (ADA);  $\beta$ -glucuronidase (GUS); lysosomal  $\alpha$ -mannosidase (MAN<sub>B</sub>); and esterase-A<sub>4</sub> (ESA<sub>4</sub>). Channels (1) RAG, mouse parental cell line; (2) ML II cultured fibroblast line GM 1006; (3) normal human fibroblasts, WI-38; (4-6) ML II  $\times$  mouse cell hybrids; (7) control man-mouse hybrids showing normal positive human enzyme expression. Control hybrids were formed with WI-38 or other normal human fibroblasts fused to RAG cells<sup>15</sup>. HYB(+) indicates hybrid clones positive for the human enzyme and HYB(-) indicates negative human enzyme expression. Arrows indicate the altered HEX, ACP<sub>2</sub>, and ADA-d, bands in ML II fibroblasts. The ML II extract for HEX was concentrated to demonstrate the altered HEX band. Differences in mobility of the rodent GUS band in hybrids is due to genetic variation in the mouse parental cells previously reported<sup>21</sup>. Starch gel electrophoretic procedures and enzyme staining were as described previously<sup>12</sup>.



by examining 35 enzyme markers representing each of the human chromosome linkage groups<sup>15,16</sup>. Although individual hybrids did not retain all human chromosomes, the entire genome was represented when all hybrids were taken together. This evidence suggests that the ML II defect was present in hybrids, but was complemented by the mouse genome.

Correction of ML II enzyme defects was also tested by examining linkage relationships of affected enzymes in cell hybrids. The negative expression of a deficient enzyme could result from either the loss of the chromosome encoding the enzyme or deficiency due to the presence of the ML II mutant gene. This was tested by examining segregation patterns of lysosomal enzymes with other enzymes assigned to the same chromosome. Concordant segregation was examined for ESA<sub>4</sub> linked to LDH<sub>A</sub> on chromosome 11 (ref. 14); HEX<sub>A</sub> linked to MPI, PK, and IDH<sub>M</sub> on chromosome 15 (refs 17, 18); and MAN<sub>B</sub> which we have assigned to chromosome 19 with GPI and PEPD (ref. 16). The ESA<sub>4</sub>, HEX<sub>A</sub>, and MAN<sub>B</sub> lysosomal enzymes in hybrids revealed normal segregation patterns with their linked markers (Table 1). Thus when the lysosomal enzyme was absent, other markers on the same chromosome were also absent. These normal segregation patterns of affected enzymes and their linked markers suggest further the correction of the ML II enzyme phenotype.

Cell hybridisation studies with other human lysosomal storage disorders have not demonstrated correction of the deficient lysosomal enzymes. Thus in Tay Sachs-rodent hybrids, the deficient human HEX<sub>A</sub> enzyme is not demonstrated<sup>5</sup>. Sandhoff-rodent hybrids do not express the deficient human HEX<sub>A</sub> and HEX<sub>B</sub> bands<sup>6,8</sup>. Fabry-hamster cell hybrids fail to express

detectable human  $\alpha$ -galactosidase<sup>7</sup>, and hybrids with mannosidosis fibroblasts do not express human lysosomal MAN<sub>B</sub> (ref. 8).

There is evidence to suggest that ML II may be associated with a deficiency in the metabolism of sialic acid coupled glycoproteins. Several lysosomal enzymes from ML II cells including HEX, ACP<sub>2</sub>, and MAN<sub>B</sub> seem to be excessively sialylated<sup>11,12</sup>. Cultured ML II fibroblasts have been reported to contain high levels of sialic acid<sup>19</sup> and are severely deficient for an acid sialidase<sup>19,20</sup>. It is not known if this sialidase deficiency represents the primary ML II lesion, however.

Complementation of ML II enzyme defects in man-mouse hybrids is indicated since (1) the enzymes HEX, ACP<sub>2</sub>, and ADA-d had normal electrophoretic patterns in ML II hybrids;

**Table 1** Segregation of lysosomal enzymes with linked enzyme markers in ML II  $\times$  mouse hybrids

	+/+	+/-	-/+	-/-
ESA <sub>4</sub> /LDH <sub>A</sub>	24	0	3	9
HEX <sub>A</sub> /MPI, PK, IDH <sub>M</sub>	20	1	0	15
MAN <sub>B</sub> /GPI, PEPD	26	1	1	8

Human gene assignments of lysosomal enzymes determined from man-rodent somatic cell hybrid studies were reported previously<sup>14-18</sup>. Values are numbers of independent hybrid clones scored positive (+) or negative (-) for expression of enzyme markers. The few discordant clones observed were similar to numbers in control hybrids and are accountable due to different sensitivities of enzyme stains and/or chromosome breakage. Enzyme markers for each hybrid clone were determined from the same cell homogenate.

(2) BGUS, MAN<sub>B</sub>, and ESA<sub>4</sub> showed normal levels of expression; (3) the ESA<sub>4</sub>, HEX<sub>A</sub>, and MAN<sub>B</sub> enzymes showed normal linkage relationships; and (4) all the human chromosomes were represented when all hybrids were considered. This evidence suggests that the ML II mutation was present and that the mouse genome can correct the multiple enzyme abnormalities of the ML II disorder.

Correction of enzyme defects in ML II hybrids shows that these enzyme abnormalities are not defects in the primary structure of individual enzymes. Rather, these results are consistent with this disorder resulting from a defect in the post-translational processing of multiple lysosomal enzymes which can be corrected by the mouse genome in man-mouse hybrids.

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1. Leroy, J. G., Spranger, J. W., Feingold, M., Opitz, J. M. Crocker, A. C. J. *Pediatr.* 79, 360–365 (1971).
2. Gilbert, E. F., Dawson, G., Zu Rhein, G. M., Opitz, J. M. & Spranger, J. W. *Z. Kinderheilkd.* 114, 259–292 (1973).
3. Leroy, J. G. *et al. Pediatr. Res.* 6, 752–757 (1972).
4. Wiesmann, U. N. & Herschkowitz, N. N. *Pediatr. Res.* 8, 865–780 (1974).
5. Shows, T. B. *Isozymes: Current Topics in Biological and Medical Research* (eds Rattazzi, M. C., Scandalios, J. G. & Whitt, G. S.) (Liss, New York, in the press).
6. Van-Cong, N., Weil, D., Rebouret, R. & Frezal, J. *Ann. hum. Genet.* 39, 111–123 (1975).
7. Hamers, M. N., Westerveld, A., Meera Khan, P. & Tager, J. M. *Hum. Genet.* 36, 289–297 (1977).
8. Champion, M. J. & Shows, T. B. *Excerpta Medica* 426, 74 (1977).
9. Hickman, S. & Neufeld, E. F. *Biochem. biophys. Res. Commun.* 49, 992–999 (1972).
10. Lie, K. K., Thomas G. H., Taylor, H. A. & Sensenbrenner, J. A. *Clin. chim. Acta* 45, 243–248 (1973).
11. Vladutiu, G. D. & Rattazzi, M. C. *Biochem. biophys. Res. Commun.* 67, 956–964 (1975).
12. Champion, M. J. & Shows, T. B. *Am. J. hum. Genet.* 29, 149–163 (1977).
13. Kelly, T. E. *Clin. Orthop.* 114, 116–136 (1976).
14. Shows, T. B. *Proc. natn. Acad. Sci. U.S.A.* 69, 348–352 (1972).
15. Lalley, P. A. *et al. Biochem. Genet.* 15, 367–382 (1977).
16. Champion, M. J. & Shows, T. B. *Proc. natn. Acad. Sci. U.S.A.* 74, 2968–2972 (1977).
17. Lalley, P. A., Rattazzi, M. C. & Shows, T. B. *Proc. natn. Acad. Sci. U.S.A.* 71, 1569–1573 (1974).
18. Solomon, E. *et al. Somatic Cell Genet.* 2, 125–140 (1976).
19. Thomas, G. H., Tiller, G. E., Reynolds, L. W., Miller, C. S. & Bace, J. W. *Biochem. biophys. Res. Commun.* 71, 188–195 (1976).
20. Strecker, G., Michalski, J. C., Montreuil, J. & Farriaux, J. P. *Biomedicine* 25, 238–240 (1976).
21. Lalley, P. A. & Shows, T. B. *Science* 185, 442–444 (1974).

## Calcium ion regulates chemotactic behaviour in bacteria

CHEMOTAXIS is the process by which bacteria travel to higher concentrations of attractant or lower concentrations of repellent. Its study has attracted wide interest as an example of the stimulus-response network since bacteria are the most primitive of living creatures. Behaviour in peritrichous bacteria is indeed simple: bacteria alternately swim smoothly and tumble, which results in random reorientation for the next swim<sup>1</sup>. Chemotaxis occurs by the increasing tendency of bacteria to tumble when headed in the 'unfavourable' direction and by increasing inclination to swim when the bacteria are headed in the 'favourable' direction (such as towards higher attractant concentrations)<sup>1,2</sup>. Tumbling is caused by clockwise rotation of flagella and swimming by counter-clockwise rotation<sup>3</sup>. Addition of repellent to bacteria causes tumbling; addition of attractant causes swimming<sup>4–6</sup>. Understanding how the switch that controls direction of flagellar rotation is controlled and how the controlling parameter is regulated by changes in attractant or

repellent concentration is necessary to understand chemotaxis. Here I present evidence that for *Bacillus subtilis* the free concentration of internal Ca<sup>2+</sup> ion controls direction of flagellar rotation (high concentrations bringing about tumbling; low concentrations, swimming) and that repellents, which cause tumbling when added to bacteria, work by increasing the flow of Ca<sup>2+</sup> ion across the plasma membrane.

*B. subtilis* was grown in minimal medium, filtered, washed and suspended at about  $1.7 \times 10^7$  bacteria per ml. A23187, an antibiotic that transports divalent cations across membranes and hence tends to equilibrate the free concentrations of these cations in aqueous compartments separated by membranes<sup>7</sup>, was sometimes added as a 1:1000 dilution from a stock in ethanol: 0.1% ethanol itself did not affect behaviour. Samples of 4 or 9  $\mu$ l were then placed, sometimes in a blind experiment, on a washed microscope slide and the bacterial behaviour observed at the top of the drop.

The purpose of the experiment was to regulate the internal concentration of Ca<sup>2+</sup> ion, which is present in very low concentrations because it is normally extruded from bacteria<sup>8</sup>, to see whether the ion might play a role in controlling behaviour. Buffered Ca was added to a basic buffer (Table 1). By adding high concentrations of the chelating agent, EGTA, and CaCl<sub>2</sub> in the correct concentrations, one could stably maintain low concentrations of free Ca<sup>2+</sup> ion. To minimise depletion of cellular Mg, EGTA was chosen because of its low stability constant for Mg<sup>2+</sup> ion ( $10^{1.9}$ ) (ref. 9).

In absence of A23187 in  $10^{-4}$ M Ca<sup>2+</sup> ion, the bacteria swam erratically<sup>10</sup>. As the concentration of free Ca<sup>2+</sup> ion was lowered, the bacteria swam progressively more smoothly. In absence of Ca<sup>2+</sup> ion, they only tumbled occasionally<sup>10</sup>. In fact, it was possible in blind experiments to distinguish, although sometimes with difficulty, the decrease in tumbling between  $10^{-4}$ M and  $10^{-5}$ M,  $10^{-5}$ M and  $10^{-6}$ M,  $10^{-6}$ M and  $10^{-7}$ M,  $10^{-7}$ M and  $10^{-8}$ M, and  $10^{-8}$ M and none.

In presence of A23187, behaviour was dramatically altered. Bacteria became incessant tumblers at  $10^{-7}$ M and higher Ca<sup>2+</sup> ion and, as without A23187, nearly incessant swimmers at  $10^{-8}$ M and lower Ca<sup>2+</sup> ion (Table 1; and see ref. 10). Furthermore, strong attractant,  $10^{-3}$ M alanine,  $10^6$ -fold above its threshold<sup>11</sup>, did not cause swimming in bacteria in  $10^{-7}$ M Ca<sup>2+</sup> ion and A23187. Finally, with no A23187, strong repellent,  $3.2 \times 10^{-6}$ M pentachlorophenol, 32-fold above its threshold<sup>6</sup> did not cause tumbling if the external Ca<sup>2+</sup> ion was  $10^{-8}$ M or lower.

These results indicate that internal Ca<sup>2+</sup> ion is likely to regulate the direction of flagellar rotation. Ca<sup>2+</sup> ion is presumed to bind, with  $K_d$  of  $10^{-7}$  to  $10^{-8}$ M, to the switch that controls

Table 1 Effect of regulation of cytoplasmic Ca<sup>2+</sup> ion using an ionophore and Ca buffer

[Ca <sup>2+</sup> ] <sub>free</sub>	[EGTA] <sub>total</sub>	[Ca <sup>2+</sup> ] <sub>total</sub>	Behaviour in A23187
$10^{-4}$	$10^{-4}$	$2 \times 10^{-4}$	Tumble
$10^{-5}$	$10^{-3}$	$9.94 \times 10^{-4}$	Tumble
$10^{-6}$	$10^{-3}$	$8.63 \times 10^{-4}$	Tumble
$10^{-7}$	$10^{-3}$	$6.13 \times 10^{-4}$	Tumble
$10^{-8}$	$10^{-3}$	$5.935 \times 10^{-4}$	Swim
0	$10^{-3}$	0	Swim

Suspension buffer contained the following components:  $5 \times 10^{-3}$ M piperazine-N, N'-bis(2-ethane sulphonate) pH 6.9,  $3 \times 10^{-2}$ M KCl, 5 mM sodium lactate, 0.05% glycerol,  $3 \times 10^{-4}$ M chelex-100 treated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and EGTA CaCl<sub>2</sub> as above. A23187 was used at  $1.37 \times 10^{-6}$ M. The concentration of free Ca<sup>2+</sup> ion was calculated from the equation<sup>21</sup>  $[EGTA]_{total} = ([Ca]_t/K[Ca^{2+}]_{free}) - 1/K + [Ca]_{total} - [Ca^{2+}]_{free}$ , using an apparent stability constant ( $K$ ) for Ca-EGTA of  $10^{6.8}$  (ref. 9). Bacteria were grown in minimal medium containing  $10^{-2}$ M Na lactate and 0.1% glycerol to 75 Klett units (filter 66), about  $2.6 \times 10^8$  bacteria per ml. Experiments were carried out as described in ref. 10 except that observation was made at the top of the drop, not near the slide, since bacteria tended to stick to the glass.



direction of flagellar rotation. When the switch is bound with  $\text{Ca}^{2+}$  ion, tumbling (clockwise rotation) is favoured; when unbound, swimming is favoured. Normally the bacteria successfully keep it out since even  $10^{-4}\text{M}$  external  $\text{Ca}^{2+}$  ion the bacteria do not tumble incessantly. But A23187 readily conveys it into the cell since even  $10^{-7}\text{M}$   $\text{Ca}^{2+}$  ion makes the bacteria perpetual tumblers. Repellent is assumed to bring about a transient depolarisation of the membrane<sup>5,6</sup> and to increase its permeability to  $\text{Ca}^{2+}$  ion since, in absence of A23187, no tumbling response ensues if the external  $\text{Ca}^{2+}$  ion concentration is low enough (see above).

Similar results were obtained in a previous study<sup>10</sup> when EGTA was added to *B. subtilis* in A23187. But, when Mg as well as Ca was removed from cells using A23187 and EDTA, then incessant tumbling ensued. One explanation<sup>12</sup> is that  $\text{Mg}^{2+}$  ion and  $\text{Ca}^{2+}$  ion are antagonists at the switch. Because internal Mg content is very high ( $20\text{--}40\text{ mM}$ )<sup>13</sup>, the fractional change in the concentration of  $\text{Mg}^{2+}$  that occurs during chemotactic stimulation is probably very small; in the absence of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  binds to the switch and the cells swim smoothly. In absence of either ion, the switch stays in the tumbling (clockwise) rotation mode.

There is strong evidence that  $\text{Ca}^{2+}$  ion has a universal role in the control of biological processes. It regulates direction of swimming by *Paramecium*, for instance, when *Paramecium* strikes a barrier, the membrane depolarises and  $\text{Ca}^{2+}$  ion enters and causes a change in the direction that the cilia point. Thus, *Paramecium* backs up<sup>14–16</sup>. (This mechanism is similar to the way repellents of *B. subtilis* act; attractants act differently<sup>12</sup>.) When light strikes rhodopsin,  $\text{Ca}^{2+}$  ion flows out of the discs in which rhodopsin is housed into the cytoplasm and there brings about depolarisation of the plasma membrane to begin transmission of the nerve impulse to the brain<sup>17</sup>. Arrival of a nerve impulse to muscle leads to release of  $\text{Ca}^{2+}$  ion from sarcoplasmic reticulum to bring about muscle contraction and its return allows muscle relaxation<sup>18</sup>. Intracellular  $\text{Ca}^{2+}$  ion, which is affected by extracellular  $\text{Ca}^{2+}$  ion, regulates insulin release from the islets<sup>19</sup>. Variations in intracellular  $\text{Ca}^{2+}$  ion, whose fluxes across the plasma membrane of liver are affected by  $\alpha$ -adrenergic hormones, regulate phosphorylase and glucose release<sup>20</sup>. This process is repeated again and again. This special regulatory role for  $\text{Ca}^{2+}$  ion may have been chosen early in evolution; it seems to be the controlling element in the most primitive of sensory systems, chemotaxis in bacteria.

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1. Berg, H. C. & Brown, D. A. *Nature* **239**, 500–4 (1972).
2. Macnab, R. M. & Koshland, D. E. *Jr Proc. natn. Acad. Sci. U.S.A.* **69**, 2509–12 (1972).
3. Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W. & Adler, J. *Nature* **249**, 74–7 (1974).
4. Tsang, N., Macnab, R. & Koshland, D. E. *Jr Science* **181**, 60–3 (1973).
5. Ordal, G. W. & Goldman, D. J. *Science* **189**, 802–5 (1975).
6. Ordal, G. W. & Goldman, D. J. *J. molec. Biol.* **100**, 102–5 (1976).
7. Reed, P. W. & Lardy, H. A. *J. biol. Chem.* **247**, 6970–7 (1972).
8. Rosen, B. P. & McClees, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 5042–6 (1974).
9. Portzehl, H., Caldwell, P. C. & Ruegg, J. C. *Biochim. biophys. Acta* **79**, 581–91 (1964).
10. Ordal, G. W. *J. Bact.* **126**, 706–11 (1976).
11. Ordal, G. W. & Gibson, K. J. *J. Bacter.* **129**, 151–5 (1977).
12. Ordal, G. W. *J. theor. Biol.* (in the press).
13. Silver, S. *Bacterial Transport* (Dekker, New York, in the press).
14. Eckert, R. *Science* **176**, 473–81 (1972).
15. Naitoh, Y. & Eckert, R. *Science* **164**, 963–5 (1969).
16. Naitoh, Y. & Kaneko, H. *Science* **176**, 523–4 (1972).
17. Hagins, W. & Yoshikami, S. *Expl Eye Res.* **18**, 299–306 (1974).

18. Van Winkle, W. B. & Schartz, A. A. *Rev. Physiol.* **38**, 247–72 (1976).
19. Gerich, J. E., Charles, M. A. & Grodsky, G. M. A. *Rev. Physiol.* **38**, 353–88 (1976).
20. Assimacopoulos-Jeannet, F. D., Blackmore, P. F. & Exton, J. H. *J. biol. Chem.* **252**, 2662–9 (1977).
21. Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. *Data for Biochemical Research* (Clarendon, Oxford, 1969).

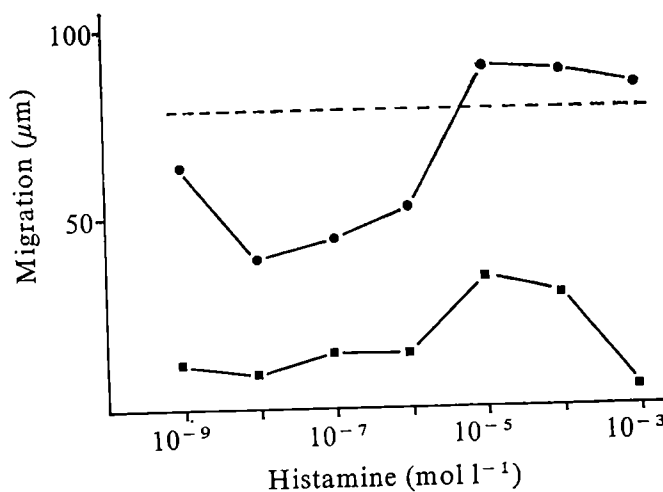
## Histamine induces release of an eosinophil immobilising factor from mononuclear cells

LIBERATION of factors chemotactic for eosinophil granulocytes is often associated with the release of histamine from mast cells and basophils. This is true for the eosinophil chemotactic factor of anaphylaxis (ECF-A), which is secreted together with histamine from mast cells and basophils<sup>1</sup>. It also applies to the generation of chemotactic factors by the activation of complement (C), which leads to the liberation of anaphylatoxin<sup>2</sup>. Therefore, it was of interest to study whether histamine had any effect on the migration of eosinophils towards chemotactic attractants. We describe here a factor which inhibits the migration of eosinophil granulocytes and which is released from mononuclear cells upon incubation with histamine.

Eosinophils were induced in guinea pigs by intraperitoneal injections of a crude extract of *Ascaris suis*, 4 mg twice weekly for at least 2 weeks. Four days after the last injection the cells were collected by peritoneal lavage. The cell suspension contained between 30 and 70% eosinophils, remainder being almost exclusively mononuclear cells. Two cell types could be separated by centrifugal elutriation with a Beckman elutriator rotor<sup>3</sup> to yield an eosinophil rich fraction of 90–97% purity, and an eosinophil poor fraction of mononuclear cells with 1–2% eosinophils.

The eosinophils showed a good chemotactic response to normal human serum which had been treated with baker's yeast (50 mg per ml serum) for 1 h at 37 °C to activate complement (C-activated NHS). They were also attracted to casein at concentrations of 0.5–2.0 mg ml<sup>-1</sup> and, to a lesser extent, to histamine, which exhibited an optimal cell response at a concentration of 10<sup>-5</sup> mol l<sup>-1</sup> (Fig. 1). To

Fig. 1 The effect of histamine at various concentrations on the migration of guinea pig eosinophils of 40% purity towards C-activated NHS. The upper compartment of the chemotaxis chamber contained  $3 \times 10^6$  cells per ml Hank's balanced salt solution (HBSS), to the lower compartment was added per ml HBSS either 50  $\mu$ l of C-activated NHS (dashed line), or histamine (■) or 50  $\mu$ l C-activated NHS plus histamine (●). Chemotaxis was assayed in a modified Boyden chamber using micropore filters of 8  $\mu$ m pore size (Schleicher & Schüll, Göttingen, Germany). The leading front of migration into the filter was measured<sup>15</sup>.



analyse the influence of histamine on the chemotactic migration towards C-activated NHS, histamine at various concentrations was added to the attractant in the lower compartment of a chemotaxis chamber. When the cell preparation contained less than 50% eosinophils, two effects were observed (Fig. 1). Concentrations of  $10^{-4}$  to  $10^{-5}$  mol l $^{-1}$ , which themselves induced chemotactic migration, increased the distance migrated by the eosinophils. At lower concentrations, however, histamine caused a dose dependent inhibition of eosinophil migration, which was most pronounced at  $10^{-8}$  mol l $^{-1}$ . In contrast, when 90% pure eosinophils were employed in the assay, only the enhancing effect at high concentrations but no inhibition of migration at low concentrations was discernable (Fig. 2). This finding suggested that histamine did not inhibit the eosinophils directly, but induced the release of an immobilising factor from cells other than eosinophils. To test this possibility further the mononuclear fraction of peritoneal cells devoid of eosinophils was incubated with and without histamine at  $10^{-8}$  mol l $^{-1}$  for 1 h at 37 °C. The cells were then centrifuged, and eosinophils of 90% purity were suspended in the supernatant and assayed for chemotactic migration towards C-activated NHS. As depicted in Fig. 3, there was some inhibition of eosinophil migration by the 1 h supernatant of mononuclear cells incubated without histamine. However, a marked inhibition of migration by 80% was found when histamine was present during the incubation. The immobilising effect could not be attributed to histamine, since histamine at  $10^{-8}$  mol l $^{-1}$  did not reduce the mobility of pure eosinophils and at higher concentrations was weakly chemotactic itself (Fig. 2).

Furthermore, the histamine antagonists diphenhydramine and metiamide (Smith, Kline and French), although capable of blocking the histamine induced release of the immobilising factor from mononuclear cells, did not affect the inhibition of eosinophil migration when added together with the mononuclear cell supernatant. The immobilising factor was not cytotoxic for eosinophils: at the end of the 2-h incubation period of eosinophils with the mononuclear cell supernatant more than 97% of the cells were found to be viable as tested by trypan blue exclusion. The effect could not be explained by the release of a chemotactic factor

Fig. 2 The effect of histamine at various concentrations on the migration of more than 90% pure eosinophils towards C-activated NHS. C-activated NHS 50  $\mu$ l per ml HBSS (dashed line), histamine only (■), C-activated NHS plus histamine (●).

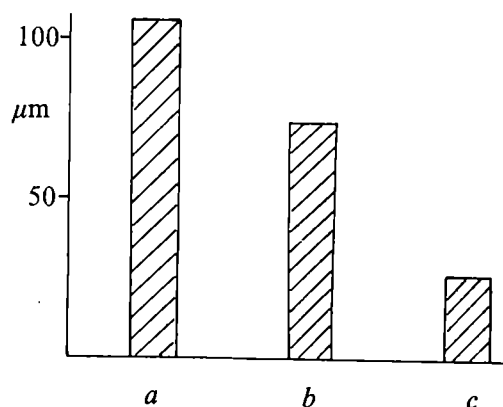
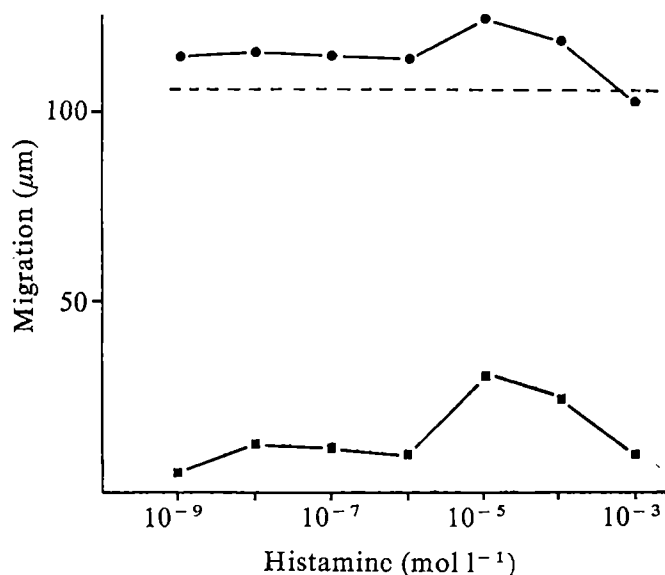


Fig. 3 The migration of 90% pure eosinophils suspended in HBSS a, the supernatant of the mononuclear cell fraction incubated for 1 h at 37 °C in the absence b and in the presence c of  $10^{-8}$  mol l $^{-1}$  histamine. The columns represent individual data from a representative experiment.

from the mononuclear cells upon histamine treatment, since the supernatant when added alone to the lower compartment of a Boyden chamber did not exhibit chemotactic activity.

We conclude therefore that histamine at a concentration around  $10^{-8}$  mol l $^{-1}$  stimulated mononuclear cells to release a factor which immobilised eosinophils. From a column of Sephadex G-10 the activity was eluted with the total volume indicating that its molecular weight was below 1,000. This precludes its identity with the neutrophil immobilising factor<sup>4,5</sup>, which has a molecular weight of 5,000. Preliminary results indicate that the eosinophil immobilising activity is released from the nonadherent fraction of mononuclear cells and that it affects the eosinophils and not the chemotactic factor.

Various effects of histamine on the function of cells have been described. Aside from an inhibitory effect on the secretory function of mast cells and basophils<sup>6,7</sup> it inhibited the destruction of target cells by cytotoxic T lymphocytes<sup>8</sup>, it reduced the number of plaque forming cells in an *in vitro* primary<sup>9</sup> and secondary<sup>10</sup> immune response, and it suppressed the production of the migration inhibitory factor MIF from immune lymphocytes<sup>11</sup>. What is unusual about the effect of histamine described here is the stimulatory action on the secretory function of mononuclear cells and the low histamine concentration at which this effect is observed.

It has been disputed whether histamine itself is chemotactic for eosinophil granulocytes. Like others<sup>12</sup> we were able to induce increased migration of eosinophils towards a concentration gradient of histamine, but this differed only slightly from the increased random mobility and was considerably less pronounced than the attraction by C-activated NHS, casein or ECF-A. It has been suggested that the chemotactic activity of histamine towards eosinophils was an *in vitro* phenomenon which could not be observed *in vivo*<sup>13</sup>. Others, being unable to detect any chemotactic attraction, nevertheless, observed immobilisation of eosinophils at the site of an injection of histamine<sup>14</sup>. It is conceivable that this effect was induced by the inhibitor of eosinophil migration described here. We suggest that the accumulation of eosinophils observed in certain allergic diseases and in nematode infections may be the result of chemotactic attraction and inhibitor-induced trapping. Furthermore, it seems possible that the inhibitory functions of histamine described by others<sup>8-11</sup> are as well mediated by

inhibitors released from mononuclear leukocytes under the influence of histamine.

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1. Kay, A. B., Stechschulte, D. J., Austen, K. F. *J. exp. Med.* 133, 602–619 (1971).
2. Müller-Eberhard, H. J. *A. Rev. Biochem.* 44, 697–724 (1975).
3. Glick, D., v. Redlich, D., Juhos, E. T. & McEwen, C. R. *Expl. cell. Res.* 65, 23–26 (1971).
4. Goetzl, E. J. & Austen, K. F. *J. exp. Med.* 136, 1564–1580 (1972).
5. Goetzl, E. J., Gigli, I., Wasserman, S. & Austen, K. F. *J. Immunol.* 111, 938–945 (1973).
6. Bourne, H. R., Melmon, K. L. & Lichtenstein, L. M. *Science* 173, 743–745 (1971).
7. Lichtenstein, L. M. & Gillespie, E. *Nature* 244, 287–288 (1973).
8. Plaut, M., Lichtenstein, L. M., Gillespie, E. & Henney, C. S. *J. Immunol.* 111, 389–394 (1973).
9. Fallah, H. A., Maillard, J. L. & Voisin, G. A. *Ann. Immunol.* 126, 669–682 (1975).
10. Melmon, K. L. *et al. J. clin. Invest.* 53, 13–21 (1974).
11. Rocklin, R. E. *J. clin. Invest.* 57, 1051–1058 (1976).
12. Clark, R. A. F., Gallin, J. I. & Kaplan, A. P. *J. exp. Med.* 142, 1462–1476 (1975).
13. Turnbull, L. W., Evans, D. P. & Kay, A. B. *Immunology* 32, 57–63 (1977).
14. Parish, W. E. *Antibiotics Chemother.* 19, 233–270 (1974).
15. Zigmond, S. H. & Hirsch, J. G. *J. exp. Med.* 137, 387–410 (1973).

## Dark noise in retinal bipolar cells and stability of rhodopsin in rods

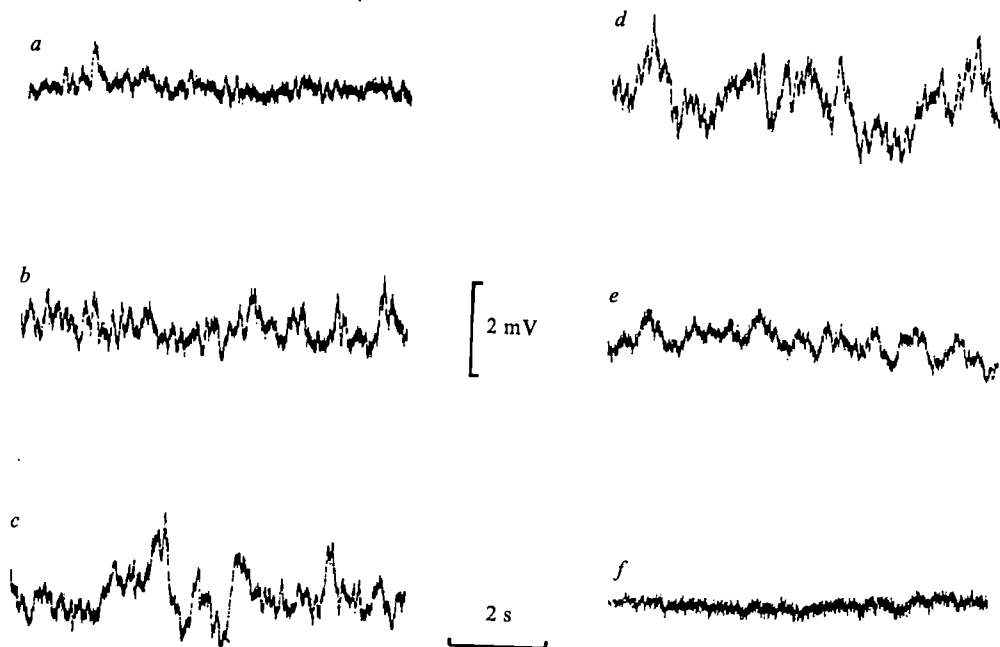
FOR the dark-adapted human observer, the absolute visual threshold has been estimated to be the effective absorption of 5–10 photons in an area covered by some 500 rods<sup>1</sup>. Although the quantum statistics of light enter as a factor which determines the frequency of seeing weak light stimuli<sup>1,2</sup>, it has been suggested that there is 'noise' in the visual system which ultimately limits the sensitivity of the eye<sup>3,4</sup>. Each rod in the human eye contains about  $10^8$  rhodopsin molecules, and if the 'noise' arises from events in the rod indistinguishable from the effects of light, there must be an extremely low probability of

spontaneous change produced in any single rhodopsin molecule (or at sites within the rod disk membrane leading to an elementary voltage change in the rod). We have analysed voltage fluctuations in bipolar cells as a probe of rod activity in the dogfish retina. A component of the noise has been identified as photon noise, superimposed on dark noise arising from photon-like events. This part of the dark noise has a large temperature dependence ( $Q_{10}$  about 8), suggesting thermal isomerisation of rhodopsin. The rate constant, extrapolated to 37 °C, would correspond to one isomerisation in 30 s in a human rod, similar to estimates from the absolute threshold for human vision.

Using intracellular microelectrodes, we have obtained recordings from bipolar cells in the dark-adapted retina of the dogfish, *Scyliorhinus canicula*. This retina contains primarily rod photoreceptors. The bipolar cells, which depolarise with light<sup>5,6</sup>, have a high flash sensitivity indicative of a large dynamic voltage gain at the rod-bipolar synapse<sup>6</sup>. Voltage fluctuations in the bipolar cells in darkness and during steady light were analysed for their power spectral distribution.

As shown in Fig. 1, the voltage noise increased from its dark level after presentation of light in the range  $2 \times 10^{-3}$  to 1 photon  $s^{-1} \mu m^{-2}$ . The power spectrum of the noise in complete darkness and during dim steady light consists of two components, one with a half-power point near 1.3 Hz and the other near 7 Hz (Fig. 2). Several features of the cell's response to light suggest that the low-frequency component of the noise arises from fluctuations in the rate of photons absorbed by the bipolar cell's rod pool ('photon noise'), superimposed on a noise present in the dark. The spectrum of the response to a dim, brief flash of light had power distributed over a similar range of frequencies as the low-frequency component of the noise spectrum (Fig. 2). At low light levels (less than 0.02 photon  $\mu m^{-2} s^{-1}$ ), the mean depolarisation produced by steady light is proportional to the light intensity. The contribution to the total noise variance, obtained from the area under the low frequency component, increases linearly with light intensity at low light levels, as expected if the underlying statistics of the source are Poisson. It was further found that the peak amplitude of the elementary event underlying the low frequency component during steady dim light was the same as that obtained from an analysis of the fluctua-

Fig. 1 Voltage fluctuations in a dogfish bipolar cell in darkness and during steady full-field illumination by blue-green light. Mean light flux density at 495 nm (photons  $s^{-1} \mu m^{-2}$ ): a, dark; b,  $1.9 \times 10^{-3}$ ; c,  $7.4 \times 10^{-3}$ ; d,  $2.1 \times 10^{-2}$ ; e, 0.92; f, 8.5. (See ref. 6 for methods of voltage recording and light calibration.) Membrane potential in the dark -47 mV; total noise variance 0.0310 mV<sup>2</sup> (corrected for electrode noise observed outside the cell). Mean depolarisation and noise variance during steady light were for each intensity illustrated (in order of increasing intensity) 0.34 mV, 0.103 mV<sup>2</sup>; 1.1 mV, 0.163 mV<sup>2</sup>; 1.8 mV, 0.227 mV<sup>2</sup>; 7.2 mV, 0.101 mV<sup>2</sup>; 9.5 mV, 0.0196 mV<sup>2</sup>. The highest intensity illustrated produced saturation of the voltage response. Peak depolarisation of the cell in response to a flash of light was 30 mV. Note that the noise variance reached a maximum value and then fell as light intensity increased. Temperature 17 °C.



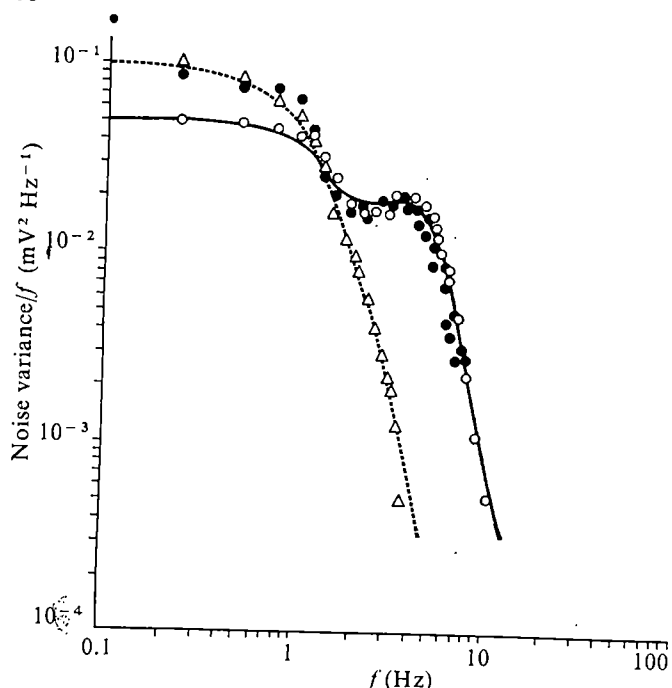


Fig. 2 Power spectra of voltage noise in darkness (○) and during steady dim light (●), compared with the spectrum of the response to a dim flash (△, the modulus squared of the Fourier transform of the flash response). Analogue data, recorded on an FM tape recorder, were filtered by a low pass filter with a cut-off frequency of 100 Hz, and sampled at 256 Hz. Spectra, weighted by a Hanning function, were computed from data blocks containing 1,024 points using the fast Fourier transform. The spectra shown are the averages obtained from 32–76 s of record and have been corrected for the electrode noise (mainly  $1/f$  noise). The spectrum of the response to a flash of light represents the average spectrum of 12 responses to 15-ms flashes delivering  $0.01 \text{ photons } \mu\text{m}^{-2}$ , which gave a mean response of 2.3 mV. The spectrum of the dark noise has been subtracted and the result arbitrarily scaled to the lowest frequency points for the spectrum in dim steady light ( $4 \times 10^{-3} \text{ photons s}^{-1} \mu\text{m}^{-2}$ , mean depolarisation 0.33 mV). Noise variance in the dark attributable to 'photon-like' events =  $0.032 \text{ mV}^2$ . Noise variance in dim steady light attributable to 'photon noise' + 'photon-like' events  $0.082 \text{ mV}^2$ . Membrane potential in the dark  $-54 \text{ mV}$ ; maximum peak depolarisation with bright light 23 mV; temperature  $23^\circ \text{C}$ .

tions in response to brief flashes of dim light<sup>7</sup>. This provides strong evidence that the same Poisson process is involved. The estimated peak amplitude of the response produced in the bipolar cell by one photon absorbed within its rod pool has an average value of 0.25 mV.

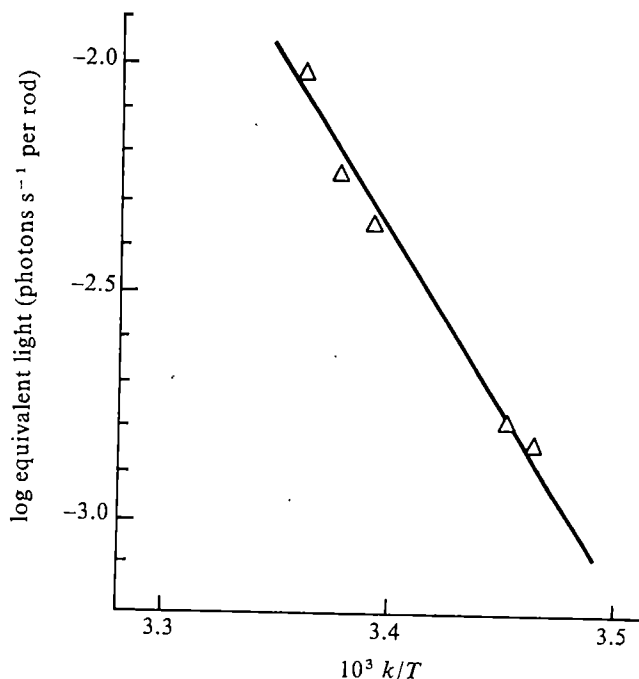
The response of photoreceptors to flashes of dim light can be described by equations of the same form as the impulse response of an electrical network consisting of a chain of low pass filters<sup>8,9</sup>. It is assumed that the narrow band character of the low frequency component of bipolar cell noise arises from the properties of the rod response rather than the filter characteristics of the rod-bipolar synapse. In that case, the low frequency component of the dark noise could arise from events in the rod indistinguishable from the effects of light. The 'dark light', which would be the photon equivalent for the low frequency component of the dark noise, can be obtained from the intercept on the light intensity axis in a plot of variance against light intensity. For the cell illustrated in Fig. 2, the 'dark light' would be equivalent to a photon flux density of  $2 \times 10^{-3} \text{ photon } \mu\text{m}^{-2} \text{ s}^{-1}$ . This would correspond to an event equivalent to one photoisomerisation in a rod every 160 s, if the effective collecting area for a dogfish rod is  $3 \mu\text{m}^2$  (ref. 6).

The 'dark light' was found to be strongly temperature dependent. An activation energy of  $36 \text{ kcal mol}^{-1}$  was

calculated from an Arrhenius plot (Fig. 3) of the mean rate of spontaneous events per rod as a function of absolute temperature, giving a  $Q_{10}$  of 7.8 at a temperature of  $20\text{--}30^\circ \text{C}$ . The activation energy associated with the dark light is significantly higher than for the processes governing the kinetics of the response of photoreceptors to flashes of light;  $10 \text{ kcal mol}^{-1}$  ( $Q_{10} = 1.8$ ) for turtle cones<sup>8</sup> and  $16 \text{ kcal mol}^{-1}$  ( $Q_{10} = 3$ ) for rat rods<sup>9</sup>. This would make it unlikely that such processes occur spontaneously at the requisite rate. If the dark light arose from spontaneous changes in rhodopsin molecules, these changes might be of two kinds, thermal denaturation or thermal isomerisation. The products of the former are denatured opsin and 11-*cis*-retinal<sup>10</sup>, whereas for the latter they are opsin and *trans*-retinal, as in photoexcitation<sup>11</sup>. The rate constant at  $20^\circ \text{C}$  would be  $1.2 \times 10^{-11} \text{ s}^{-1}$ , based on Fig. 3 and an estimate of  $3 \times 10^8$  rhodopsin molecules per dogfish rod. Applying absolute reaction rate theory<sup>12</sup>, we find that the Gibbs free energy of activation  $\Delta G^\ddagger$  for the process underlying the dark light is  $32 \text{ kcal mol}^{-1}$ , and the entropy of activation  $\Delta S^\ddagger$  is  $13 \text{ cal mol}^{-1} \text{ degree}^{-1}$ . The entropy of activation is very much less than for thermal denaturation of rhodopsin<sup>10</sup>. We conclude, therefore, that denaturation occurs at a comparatively insignificant rate in the eye over the temperature range of  $15\text{--}25^\circ \text{C}$  and/or it does not give rise to responses in rods which resemble photoreponses.

The activation energy of  $36 \text{ kcal mol}^{-1}$ , determined from the dark noise, lies between the value of  $26 \text{ kcal mol}^{-1}$  for thermal *cis-trans* isomerisation of the chromophore, retinal, in a non-polar solvent, as determined by Hubbard<sup>13</sup>, and an estimate of about  $48 \text{ kcal mol}^{-1}$  for the activation energy for thermal isomerisation of rhodopsin<sup>14,15</sup>. The estimate of  $48 \text{ kcal mol}^{-1}$  for rhodopsin is not based on direct measurement but derives from the assumption that there is a fixed minimum energy required for absorption of

Fig. 3 Arrhenius plot of the dark rate of spontaneous events in a dogfish rod, equivalent to photoisomerisation, against the reciprocal of the absolute temperature. The ordinate is the log of the mean rate of effective photon absorption by rods which would be required in order to give 'photon' noise variance in the bipolar cell equal to the variance in the dark associated with the low frequency component of the noise (see text). An effective collecting area of  $3 \mu\text{m}^2$  for a rod in the tapetal region of the retina has been assumed<sup>6</sup>. The slope of the line corresponds to an Arrhenius activation energy of  $36 \text{ kcal mol}^{-1}$ .



a photon<sup>15-18</sup>, which would correspond to the thermal activation energy for isomerisation. This would, however, predict a very much larger energy of activation for *cis-trans* isomerisation of retinal than was observed by Hubbard<sup>21</sup> (see also ref. 18). We conclude tentatively that the present results are consistent with thermal isomerisation of rhodopsin. Extrapolation to 37 °C would give a rate constant of  $3.5 \times 10^{-10} \text{ s}^{-1}$ . Denton and Pirenne<sup>3</sup>, and Barlow<sup>4</sup>, have concluded that in the human the rate constant for thermal isomerisation of rhodopsin must be less than  $10^{-9} \text{ s}^{-1}$  in order to account for the reliability of detection at absolute threshold. The low rate of spontaneous isomerisation of rhodopsin is one of the features of the visual system underlying the ability to detect the absorption of a few photons.

It is not yet clear whether the second component of the noise in bipolar cells reflects synaptic noise arising from fluctuations in the release of transmitter at the rod-bipolar synapse<sup>19</sup> or noise generated more distally in the photoreceptor, possibly from interaction of an intracellular transmitter with the surface membrane of the rod<sup>20-22</sup>.

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- Hecht, S., Schlaer, S. & Pirenne, M. H. *J. gen. Physiol.*, **25**, 819-840 (1942).
- Rose, A. *J. opt. Soc. Am.*, **38**, 196-208 (1948).
- Denton, E. J. & Pirenne, M. H. *J. Physiol., Lond.*, **123**, 417-442 (1954).
- Barlow, H. B. *J. opt. Soc. Am.*, **46**, 634-639 (1956).
- Ashmore, J. F. & Falk, G. *J. Physiol., Lond.*, **258**, 39-40P (1976).
- Ashmore, J. F. & Falk, G. *Nature*, **263**, 248-249 (1976).
- Ashmore, J. F. & Falk, G. *J. Physiol., Lond.*, **269**, 27-28P (1977).
- Baylor, D. A., Hodgkin, A. L. & Lamb, T. D. *J. Physiol., Lond.*, **242**, 685-727 (1974).
- Penn, R. D. & Hagins, W. A. *Biophys. J.*, **12**, 1073-1094 (1972).
- Hubbard, R. *J. gen. Physiol.*, **42**, 259-280 (1958).
- Hubbard, R. & Kropf, A. *Proc. natn. Acad. Sci. U.S.A.*, **44**, 130-139 (1958).
- Glasstone, S., Laidler, K. J. & Eyring, H. *The Theory of Rate Processes* (McGraw Hill, New York, 1941).
- Hubbard, R. *J. biol. Chem.*, **241**, 1814-1818 (1966).
- St. George, R. C. *J. gen. Physiol.*, **35**, 495-517 (1952).
- Dartnall, H. J. A. *The Visual Pigments* (Methuen, London, 1957).
- Stiles, W. S. in *Transactions of the Optical Convention of the Worshipful Company of Spectacle Makers* 97-107 (Spectacle Makers' Company, London, 1948).
- Denton, E. J. & Pirenne, M. H. *J. Physiol., Lond.*, **125**, 181-207 (1954).
- de Vries, H. *Progr. Biophys. biophys. Chem.*, **6**, 207-264 (1955).
- Falk, G. & Fatt, P. in *Handbook of Sensory Physiology* 7 (ed. Dartnall, H. J. A.) 200-244 (Springer, Heidelberg, 1972).
- Simon, E. J., Lamb, T. D. & Hodgkin, A. L. *Nature*, **256**, 661-662 (1975).
- Simon, E. J. & Lamb, T. D. in *Vertebrate Photoreception* (ed. Barlow, H. B. & Fatt, P.) (Academic, London, in the press).
- Schwartz, E. A. in *Vertebrate Photoreception* (ed. Barlow, H. B. & Fatt, P.) (Academic, London, in the press).

## Acetylcholine receptor conformational transition on excitation masks disulphide bonds against reduction

KARLIN and Bartels<sup>1</sup> found that dithiothreitol (DTT) inhibits the responses induced by acetylcholine (ACh) in the *Electrophorus electricus* electroplex preparation and that 5,5'-dithio-bis-(2-nitrobenzoic acid) completely restored the membrane sensitivity to ACh. These results illustrate the importance of disulphide bonds for acetylcholine receptor (AChR) function, and work on other preparations with nicotinic AChRs gave similar results<sup>2-9</sup>. Several facts suggest that DTT affects AChRs specifically; (1), the significance of the quaternary ammonium group in the molecules of alkylating and acylating agents for the rate of their reaction with the AChR reduced by DTT<sup>10</sup>; (2) changes in pharmacological specificity seen in the modified receptor<sup>5,7,10</sup>; (3) the decrease in the slope of the dose-response curve to carbamylcholine expressed as the Hill plot<sup>11</sup>; and (4) the increase in *d*-tubocurarine affinity to the AChR active site<sup>5</sup>. If we could protect the receptor against chemical modification by an agonist or antagonist, this would be

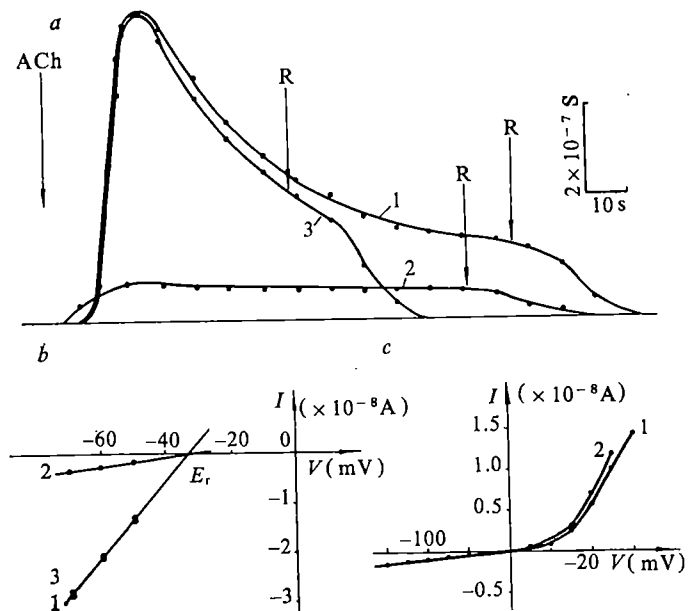


Fig. 1 The effect of disulphide bond reduction by DTT and reoxidation by DTBCh on soma membrane of *Limnaea stagnalis* neurone. 1, Control; 2, after 14 min treatment with DTT  $1 \times 10^{-3} \text{ M}$ ; 3, after 15 min reoxidation by DTBCh  $1 \times 10^{-4} \text{ M}$ . *a*, The time course of membrane conductance change induced by ACh  $2 \times 10^{-6} \text{ M}$ . The times of ACh and Ringer solution (R) addition are marked by arrows. *b*, Voltage-current plots of the neurone response to ACh  $2 \times 10^{-6} \text{ M}$ . The reversal potential,  $E_r$  (obtained by extrapolation), does not change after the neurone exposure to DTT. *c*, The steady-state voltage-current curves of electroexcitable membrane. In (*a*) and (*b*) membrane potential was held at the resting potential (RP) level and a three-level voltage sequence (RP + 10 mV, RP, RP - 10 mV) was applied. ChRM conductance values were calculated according to current changes produced by membrane potential shifts in equal periods of time (dots in (*a*) and in Figs. 2 and 3). Curves were drawn by hand. In (*c*) membrane potential was held at RP level and a serial depolarising and hyperpolarising voltage steps (10 mV) were applied. Impulse duration, 5 s. Potassium outward currents were measured at the end of the steps.

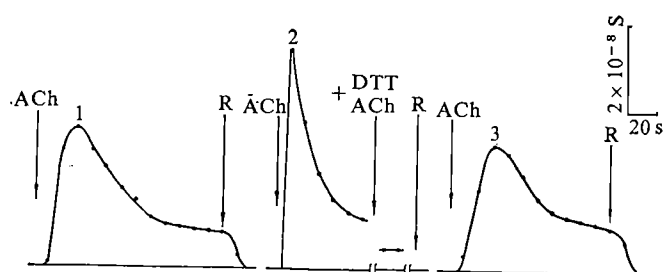
the most reliable proof of the specificity of modifying agent action<sup>12</sup>. We present here a detailed study of the action of DTT on nicotinic AChRs of completely isolated *Limnaea stagnalis* neurones in order to clarify the ability of various cholinergic ligands to protect the receptor disulphide bonds against reduction by DTT.

Identified giant neurones from the right and left parietal ganglia of molluscs were isolated by treating brain preparations with trypsin and then Pronase, and separated mechanically with tungsten microneedles and glass micropipettes<sup>13,14</sup>. Nerve cells were placed into the chamber (effective perfusion volume 0.2 ml, rate of perfusion 15-20 ml per min) and continuously perfused with *Limnaea* Ringer solution containing NaCl 100 mM, KCl 1.6 mM, CaCl<sub>2</sub> 4 mM, MgCl<sub>2</sub> 1.5 mM, Tris-hydroxymethylaminomethane 0.12 g l<sup>-1</sup>, and HCl to pH 7.5. All the drugs were dissolved in this medium and applied to the neurone membrane by passing constant-flowing (gravity feed) solution. Each neurone was impaled with two microelectrodes (2.5 M KCl filled). The perfusing medium was connected with the virtual ground of an operational amplifier through the agar bridge. Drug action was estimated using the voltage-clamp technique according to the membrane conductance change at the resting potential level.

Application of DDT  $1 \times 10^{-4}$ - $1 \times 10^{-3} \text{ M}$  for 2-15 min markedly depressed responses to a standard ACh concentration (Fig. 1*a*), while the reversal potential of ACh-responses and the steady-state current-voltage curve of electroexcitable membrane did not change (Fig. 1*b* and *c*, respectively). The sensitivity of neurone membrane to ACh was restored after treatment with dithiobischole<sup>19</sup> (DTBCh) which is known to oxidate thiol groups (Fig. 1*a*, *b*).

The reversible inhibition of cholinergic membrane





**Fig. 2** Protection of ChRM against the DTT effect by a high ACh concentration. 1 And 3, response of the neurone to ACh  $2 \times 10^{-6}$  M before and after exposure to DTT  $1 \times 10^{-3}$  M for 2 min in the presence of ACh  $5 \times 10^{-5}$  M. 2, Response to conditioning ACh concentration; the duration of pretreatment with ACh was about 2 min.

(ChRM) by DTT could be converted into irreversible inhibition by application of an SH-specific alkylating agent (such as *N*-ethylmaleimide (NEM), any quaternary ammonium *N*-alkyl derivative of maleimide, or bromoacetylcholine (BrACh)) on the neurone. On the native neurone BrACh caused a strong reversible increase in membrane conductance, being only 3–5 times less active than ACh; quaternary ammonium *N*-alkyl derivatives of maleimide were very weak agonists (3–4 orders less effective than ACh) and NEM did not excite ChRM at all. It was not possible to restore the ACh-sensitivity to the initial level with DTBCh after application of an alkylating agent to DTT-treated neurone membrane. These results underline the important role of disulphide bonds in the ACh-induced conductance changes of neurone membrane which has been shown for both electroplax and skeletal muscle preparations.

BrACh seems to cause the irreversible depolarisation of electroplax membrane pretreated with DTT<sup>15</sup>, but this has not been observed with *Aplysia* neurones<sup>9</sup> and we have never observed this effect with *Limnaea* neurones. In addition, the responses of neurone membrane to ACh and BrACh decreased approximately to the same extent after DTT pretreatment. These data suggest that BrACh cannot activate reduced AChRs of mollusc neurone membrane.

We have tested cholinergic antagonists and agonists for the ability to protect ChRM against DTT-induced modification. The specific competitive nicotinic antagonist, *d*-tubocurarine ( $1 \times 10^{-3}$  M) completely blocked response to supramaximal ACh concentrations (up to  $1 \times 10^{-4}$  M) but did not prevent the modification of ChRM by DTT. In contrast to this unexpected finding high ACh concentrations effectively protected ChRM against the action of DTT (Fig. 2).

One explanation of the difference in *d*-tubocurarine and ACh protecting abilities may be that the molecules of these two cholinergic agents interact with different sites in AChR so that ACh produces a steric hindrance for DTT attack on disulphide bond, but *d*-tubocurarine does not. Alternatively, binding of ACh molecule to the AChR active site might evoke a conformational transition of AChR which would protect the disulphide bond from reduction; binding of *d*-tubocurarine would not induce such a conformational change.

In order to test the first hypothesis we compared the protecting ability of two pairs of drugs: tetramethylammonium (TMA)–phenyltrimethylammonium (PhTMA) and decamethonium–diphenyldecamethonium. The first agent of each pair is a cholinergic agonist, the second drug (a structural analogue of the other) does not excite but blocks the receptors effectively. Neither PhTMA nor diphenyldecamethonium protected ChRM against DTT-reduction, while their exciting analogues, TMA and decamethonium, partially blocked the effect of DTT (Table 1). It seems highly unlikely that the drugs with such similar chemical structures as TMA and PhTMA or decamethonium and diphenyldecamethonium should bind to different sites on AChR. A direct correlation between the activity of agonists and the ability to prevent the modifying action of DTT was found (Table 1). ACh,

**Table 1** Effect of cholinergic ligands on AChR inactivation induced by DTT

Ligand	Relative efficiency of exciting action*	Concentration used to protect against DTT (M)	Extent of protection†	Relative efficiency of protection against ‡
ACh	1	$1 \times 10^{-5}$	0.60 (5)	1
TMA	0.02	$1 \times 10^{-4}$	0.64 (3)	0.107
Decamethonium	0.0014	$1 \times 10^{-4}$	0.28 (4)	0.047
PhTMA	0.0001	$1 \times 10^{-3}$		
Diphenyl-decamethonium	0	$5 \times 10^{-3}$	0 (6)	0
<i>d</i> -Tubocurarine	0	$1 \times 10^{-3}$	0 (2)	0
		$1 \times 10^{-3}$	0 (4)	0

\* Calculated by the ratio of the equieffective concentrations relying upon increase in ChRM conductance.

† Calculated by the formula  $(c-b)/(a-b)$  where  $a$  = response to a standard ACh concentration in control;  $b$  = response to the same ACh concentration after DTT treatment;  $c$  = response to standard ACh concentration after treatment with DTT + protector. No. of experiments given in parenthesis.

‡ Calculated by the ratio of protection extent and protector concentration.

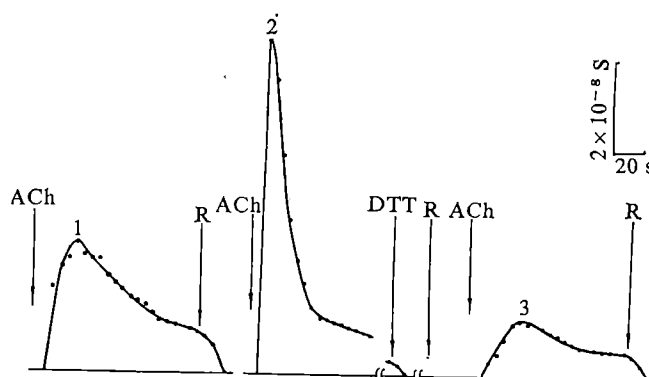
TMA and decamethonium therefore seem to protect AChRs by inducing a transition in AChR to another conformation when the agonist binds with the active site; in the new conformation the disulphide bond becomes inaccessible to DTT action.

Thus our results suggest that it is the receptor molecule alone that DTT modifies to cause inactivation of ChRM.

In all experiments involving the use of agonists as protectors DTT was added into the perfusing medium when almost all the AChRs were desensitised (Fig. 2). Presumably the AChR active site conformational change protecting the disulphide bond against reduction corresponds to the desensitisation but not to the excitation. This suggestion seems quite likely according to data on conformational transition of AChRs revealed by fluorescent dyes in membrane fragments of electric organs<sup>16,17</sup> in the presence of high (desensitising) agonist concentrations. To test this possibility we compared the DTT action in the presence and absence of ACh after 2 min exposure of the neurone to a high ACh concentration. In the latter case ACh was washed out with physiological solution containing DTT when desensitisation was complete (Fig. 3). A considerable fraction of AChRs was shown to be still in desensitised state during the first 1.5–2 min after ACh was removed from the perfusing solution. In these conditions DTT inactivated ChRM as strongly as it did in the complete absence of a protector. Apparently, for successful protection, the AChR–agonist complex must exist.

We therefore conclude first, that AChR does not lose its affinity

**Fig. 3** Inactivation of ChRM by DTT treatment immediately after removal of ACh ( $1 \times 10^{-5}$  M) from the perfusing solution. Control experiments ensured that responses to a standard ACh concentration were very much reduced in 1.5–2 min after washout of ACh  $1 \times 10^{-5}$  M (a result of desensitisation). 1 And 3, Responses to ACh  $2 \times 10^{-6}$  M; 2, response to conditioning ACh concentration ( $1 \times 10^{-5}$  M).



to agonists in the desensitised state; and second, the active site of the receptor in both the activated (non-desensitised) and the desensitised states can experience a conformational transition when interacting with an agonist molecule; the disulphide bond is hidden as a result of this process.

Desensitisation of *Limnaea* neurone membrane has been shown not to be due to changes in the system of ionic transport through the excited ChRM<sup>18</sup>. Considering these data and our results we suppose that the desensitisation of *Limnaea* neurone membrane is due to the specific state of a unit coupling the excited active site of AChR and ChRM ionophore.

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1. Karlin, A. & Bartels, E. *Biochim. biophys. Acta* **126**, 525–535 (1966).
2. Albuquerque, E. X., Sokoll, M. D., Sonesson, B. & Thesleff, S. *Eur. J. Pharmac.* **4**, 40–46 (1968).
3. Mittag, T. W. & Tormay, A. *Fedn Proc.* **29**, 574 (1970).
4. Del Castillo, J., Escobar, I. & Gijon, E. *Int. J. Neurosci.* **1**, 199–209 (1971).
5. Rang, H. P. & Ritter, J. M. *Molec. Pharmac.* **7**, 620–631 (1971).
6. Eldefrawi, M. E. & Eldefrawi, A. T. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1776–1780 (1972).
7. Ross, D. H. & Triggle, D. J. *Biochem. Pharmac.* **21**, 2533–2536 (1972).
8. Ben-Haim, D., Landau, E. M. & Silman, I. *J. Physiol. Lond.* **234**, 305–325 (1973).
9. Sato, T., Sato, M. & Sawada, M. *Jap. J. Physiol.* **26**, 471–485 (1976).
10. Karlin, A. & Winnik, M. *Proc. natn. Acad. Sci. U.S.A.* **60**, 668–674 (1968).
11. Karlin, A. *J. theor. Biol.* **16**, 306–320 (1967).
12. Singer, S. J. *Adv. Protein Chem.* **22**, 1–54 (1967).
13. Kostenko, M. A. *Cytologia* **14**, 1274–1279 (1975).
14. Kostenko, M. A., Geletyuk, V. I. & Veprintsev, B. N. *Comp. Biochem. Physiol.* **49A**, 89–100 (1974).
15. Silman, I. & Karlin, A. *Science* **164**, 1420–1421 (1969).
16. Schimmler, M. & Raftery, M. A. *Biochem. biophys. Res. Commun.* **73**, 607–613 (1976).
17. Grünhagen, H. H. & Changeux, J.-P. *J. molec. Biol.* **106**, 517–535 (1976).
18. Bregestovski, P. D., Vulfius, C. A. & Veprintsev, B. N. in *Acetylcholine Receptor Function and the Structure of its Active Site* 113–139 (Pushchino, 1975).
19. Bartels, E., Deal, W., Karlin, A. & Mautner, H. *Biochim. biophys. Acta* **203**, 568–571 (1970).

## Cellular regulation of an allosteric modifier of fish haemoglobin

ADENOSINE TRIPHOSPHATE (ATP) is the major organic phosphate in the red blood cells of a wide variety of fish species<sup>1–4</sup>. Its role as an allosteric modifier of fish haemoglobins is similar to that of 2,3-diphosphoglycerate (2,3-DPG) in mammalian red blood cells, in that it decreases the affinity of haemoglobin for oxygen<sup>5,6</sup>. After exposure to the anoxia of high altitude or in certain conditions of anaemia, mammals increase red blood cell 2,3-DPG to facilitate oxygen unloading at the tissues<sup>7</sup>. By contrast, eels have been shown to decrease erythrocyte organic phosphate (that is, ATP) and increase haemoglobin–oxygen (Hb–O<sub>2</sub>) affinity when acclimated to low environmental oxygen<sup>8</sup>. To test if this phenomenon was expressed in another fish species, we acclimated *Fundulus heteroclitus*, a euryhaline minnow, to hypoxic conditions. Our finding that this fish also lowered red blood cell ATP by as much as 40% (Fig. 1) suggests that this is a general response to hypoxia among water-breathing vertebrates.

The mechanism by which hypoxic fish lower red blood cell ATP *in vivo* is unknown. We predicted that if the control mechanism was directed at the erythrocyte level, then fish red blood cells should decrease ATP, *in vitro*, in anoxic conditions. Consistent with our *in vivo* observation, we found that anaerobic *F. heteroclitus* red cells significantly lowered their ATP levels *in vitro* (Fig. 2). Since the nucleated erythrocytes of fish possess mitochondria, we reasoned that this response may be mediated by way of a decrease in oxidative phosphorylation. This hypothesis was supported when aerated cells were incubated in the presence of low concentrations of cyanide. This inhibitor of aerobic respiration reduced intracellular ATP to levels similar to those found in the anoxic cells (Fig. 3). Thus, it seems that one reason fish have retained a functional oxygen-consuming electron transport system in the

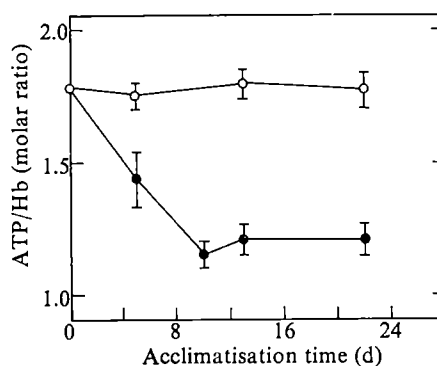


Fig. 1 Time course of acclimatization of *F. heteroclitus* to hypoxic (●) and normoxic (○) conditions at 22 °C. Dissolved oxygen values were 1.5–2.0 p.p.m. for hypoxic and 8.5–9.0 p.p.m. for control fish. Red blood cell ATP for this experiment and all others described in this paper were determined using the firefly luciferase assay<sup>9</sup>. All points represent averages of 6–7 fish. Bars indicate  $\pm$ s.e.m.

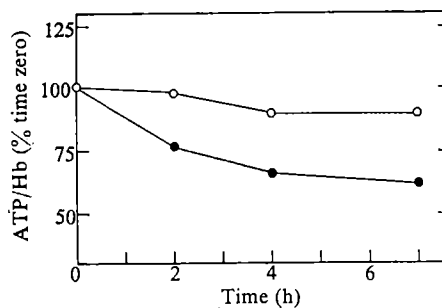
mitochondria of their red blood cells is to control the levels of haemoglobin allosteric effector.

Glycolytic inhibition experiments are consistent with the cyanide data (Fig. 3). With iodoacetate, an inhibitor of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), reducing equivalents from glycolysis are not available for oxidation by the mitochondrial electron transport chain and less pyruvate is oxidised in the tricarboxylic acid (TCA) cycle. Consequently, red blood cells exposed to this inhibitor lower their ATP to levels nearly equal to those of cyanide-poisoned cells. In the presence of fluoride, which inhibits enolase, the red blood cells show concentrations of ATP only slightly lower than control levels since reducing equivalents are formed during the earlier GAPDH reaction which can then fuel mitochondrial oxidative phosphorylation.

Assuming that iodoacetate and fluoride are acting as specific inhibitors in the red blood cells, these data indicate that the small amount of reducing equivalents from glycolysis would be nearly identical to that resulting from a fully functioning TCA cycle. Since no data exist on the relative contributions of glycolysis and the TCA cycle to the production of reduced nicotinamide adenine dinucleotide in fish erythrocytes, one cannot be certain of the intracellular potential to oxidise pyruvate. In fact, the information reported here suggests that the TCA cycle may play a substantially smaller role in fish red blood cell mitochondria compared with other tissues. We are currently investigating this possibility.

Since a physical dependence exists between oxygen solubility and water temperature (oxygen concentration is 12.5 p.p.m. at 10 °C compared with 7.5 p.p.m. at 30 °C), our observation that *F. heteroclitus* lowers red blood cell ATP when acclimated to elevated temperatures (group I, Table 1) prompted us to ask if this response was triggered by reduced oxygen (as in our hypoxic experiments), increased temperature, or both of these variables. Therefore, fish were acclimated to various temperatures (10 °C, 22 °C, and 30 °C), but with oxygen concentration maintained

Fig. 2 ATP/Hb molar ratio in *F. heteroclitus* red blood cells incubated in anaerobic (●) and aerobic (○) conditions. Cells were washed three times in PBS (pH 7), and then maintained in a medium containing physiological concentrations of inorganic salts and glucose buffered at pH 7.5 as described elsewhere<sup>9</sup>. Values are from duplicate determinations which did not vary by more than 6%.



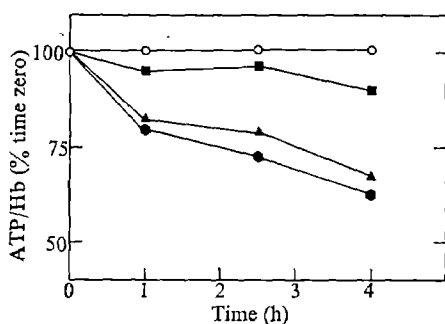


Fig. 3 ATP/Hb molar ratio in fish red blood cells incubated in media at pH 7.5 with no inhibitor (○),  $10^{-2}$  M NaF (■),  $5 \times 10^{-4}$  M iodoacetate (▲) or  $10^{-4}$  M NaCN (●). Values are from duplicate determinations which did not vary by more than 5%.

constant (about 7 p.p.m.) at each temperature. These animals also decreased their erythrocyte ATP with increased temperature (group II, Table 1). Moreover, fish acclimatised to 10 °C, but in air saturated water (12.5 p.p.m.), showed the same red blood cell ATP levels as those maintained at 10 °C but with 7 p.p.m. oxygen (Table 1). These data demonstrate that the ATP response was elicited by increased temperature alone and was independent of dissolved oxygen levels in the range 7–12.5 p.p.m.

Table 1 Comparison of red blood cell ATP/Hb molar ratios in *F. heteroclitus* acclimatised to different temperatures

Temperature (°C)	Group I	Group II
10	1.84 ± .05	1.81 ± .07
22	1.68 ± .06	1.72 ± .08
30	1.31 ± .08	1.29 ± .06

In group I, dissolved oxygen values at the various temperatures were: 10 °C, 12.5 p.p.m.; 22 °C, 9.0 p.p.m.; and 30 °C, 7.5 p.p.m. The dissolved oxygen for group II was maintained at a constant value of 7.0 p.p.m. during the course of the acclimatisation (4 weeks). All values represent averages of 10–12 fish. Errors are reported as  $\pm$  s.e.m.

As outlined earlier, there is a significant ATP response when isothermal hypoxia is induced (that is, below 3 p.p.m.). Under hypoxia, *F. heteroclitus* also increased the number of circulating red blood cells (haematocrit) by 40%. This phenomenon has been reported and discussed previously for other fish species<sup>8</sup>. In an earlier study, we reported a significant increase in the haematocrit of warm-acclimatised *F. heteroclitus*<sup>11</sup>. In the present study, however, we found no difference in haematocrit between fish acclimatised to different temperatures. In addition, red blood cell ATP concentrations of 30 °C-acclimatised fish (see Table 1) were higher than those described previously<sup>11</sup>. To simulate natural conditions, oxygen levels were not controlled in our earlier study. Therefore, biological oxygen demand (microbial activity, and so on) at the higher temperature probably lowered dissolved oxygen into the hypoxic range. In these conditions, which *F. heteroclitus* often encounter in their natural habitat, temperature and oxygen act in tandem to control red blood cell organic phosphate and, in turn, Hb-O<sub>2</sub> affinity. When governed solely by its physical dependence on temperature, however, dissolved oxygen never reaches the low values required to induce hypoxia.

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- Gillen, R. G. & Riggs, A. *Comp. Biochem. Physiol.* **38B**, 585–595 (1971).
- Granet, M. A., Weiss, M. B. & Powers, D. A. *Biol. Bull.* **145**, 437 (1973).
- Powers, D. A., Granet, M. A. & Greaney, G. *Fedn Proc.* **33**, 1302 (1974).
- Powers, D. A. *Ann. N.Y. Acad. Sci.* **241**, 472–490 (1974).
- Gillen, R. G. & Riers, A. *J. biol. Chem.* **247**, 6039–6046 (1972).

- Powers, D. A. & Edmundson, A. B. *J. biol. Chem.* **247**, 6686–6693 (1972).
- Lenfant, C., Torrance, J. D., Woodson, R. & Finch, C. A. in *Red Cell Metabolism and Function* (203–211) (Plenum, New York, 1970).
- Wood, S. C. & Johansen, K. *Nature* **237**, 278–279 (1972); *Neth. J. Sea Res.* **7**, 328–338 (1973).
- Greaney, G. S. & Powers, D. A. *J. exp. Zool.* (in the press).
- Powers, D. A. & Powers, D. in *Isozymes* **4**, 63–84 (Academic, New York, 1975).

## Thyroidal trophic influence on skeletal muscle myosin

CROSS-INNervation experiments have convincingly demonstrated the importance of the nerve in determining the differential properties of skeletal muscle myosin. The role of hormones in deciding the phenotypic character of muscle myosin has received considerably less attention than that of the neural factors. Recent studies have shown that short-term alterations in thyroid status results in significant changes in cardiac muscle myosin<sup>1–4</sup>. In contrast, some of those investigations also used skeletal muscle, but no apparent effects on skeletal muscle myosin were detected<sup>2,4</sup>. But the greater half-life of skeletal muscle myosin would require a longer time than cardiac myosin to manifest these changes<sup>5</sup>. We have studied the effects of longer term hypothyroidism and hyperthyroidism on skeletal muscle myosin. We report here that alterations in thyroid status influence the quality of skeletal muscle myosin. Our observations suggest possible thyroidal involvement, along with or through neural trophic factors, in determining the phenotypic properties of skeletal muscle myosin.

Normal, hypothyroid and hyperthyroid male Sprague-Dawley albino rats with initial body weights of 180–200 g were subjects. Thyroidectomised animals were purchased from Hormone Assay (Chicago). Hyperthyroidism was induced in thyroidectomised rats by subcutaneous injections of 30 µg per 100 g body weight of Na 3,3',5 triiodo-L-thyronine on alternate days for six weeks. Animals were killed by exsanguination under pentobarbital anaesthesia (60 mg per kg). Thyroid status of the animals was confirmed by the enzyme activities of  $\alpha$ -glycerolphosphate dehydrogenase (EC 1.1.99.5)<sup>6</sup> and succinate dehydrogenase (EC 1.3.99.1)<sup>7</sup> from whole homogenates of liver and soleus muscle at 25 °C (Table 1).

Immediately after excision a mid-portion of the soleus muscle was prepared for histochemistry. The tissue was mounted and frozen in isopentane cooled with liquid nitrogen. Cross-sections of 10-µm thickness were cut at –20 °C and stained for myofibrillar adenosine triphosphatase (M-ATPase). The staining procedure involved 10 min pre-incubation (37 °C) in glycine or 2-amino-2-methyl-1-propanol buffer (pH 10.30) containing 18 mM CaCl<sub>2</sub> followed by 30 min incubation in pre-incubation medium containing 3.08 mM ATP and adjusted to pH 9.40. This is essentially the method of Padykula and Herman<sup>8</sup> as modified by Guth and Samaha<sup>9</sup>. The difference in staining intensity is based on the alkali stability and lability of fast and slow skeletal muscle myosin ATPase activity, respectively<sup>9,10</sup>, resulting in a more intense stain for fast than slow muscle fibres at an alkaline pH.

Soleus muscles used for myosin isolation were pooled into groups of four and stored in glycerol at –20 °C. Myosin was prepared using the method of Bárány and Close<sup>11</sup>. The yield of myosin protein was similar for each experimental group (Table 1). Mg<sup>2+</sup>-activated ATPase activity indicated minimal actin contamination (1.06 nmol per mg protein per min). Ca<sup>2+</sup>-stimulated myosin ATPase activity was determined at 37 °C at various pH values using the reaction mixture of Bárány and Close<sup>11</sup> and the inorganic phosphate assay of Rockstein and Herron<sup>12</sup>. Protein determinations were by the Lowry method with bovine albumin as the standard<sup>13</sup>.

Histochemical observations indicate that thyroid status

Table 1 Effects of thyroid status on soleus muscle myosin

	Euthyroid	Hypothyroid	Hyperthyroid
Final body weight (g)	385±6 (14)	297±6 (14)*	242±8 (8)*
Liver			
SDH activity	24.86±0.79 (11)	18.07±0.84 (11)*	36.10±3.29 (7)*
GPDH activity	4.65±0.18 (11)	3.18±0.10 (12)*	11.80±0.58 (8)*
Protein (mg g <sup>-1</sup> )	216±9 (14)	210±10 (13)	226±9 (8)
Soleus muscle			
Wet weight (mg)	137±3 (28)	120±4 (28)†	119±2 (16)†
SDH activity	3.07±0.08 (18)	1.46±0.03 (14)*	4.70±0.07 (12)*
GPDH activity	1.47±0.04 (18)	1.11±0.02 (14)*	2.58±0.09 (12)*
Protein (mg g <sup>-1</sup> )	185±10 (15)	188±8 (13)	186±7 (14)
Myosin yield (mg g <sup>-1</sup> )	31.99±3.84 (7)	34.36±2.75 (7)	33.16±2.94 (4)
Alkali-stable fibres (%)	15.95±1.40 (8)	0.12±0.07 (9)*	35.09±2.03 (6)*

Values are means ± s.e.m. The number in parenthesis is the number of analyses. SDH, succinate dehydrogenase. GPDH, α-glycerolphosphate dehydrogenase. Enzyme activities are in μmol per g wet wt per min.

\*Statistically significant at  $P < 0.001$ .

†Statistically significant at  $P < 0.01$ .

can alter the proportion of alkali stable (fast) and labile (slow) M-ATPase fibres in rat skeletal muscle (Fig. 1). The soleus muscles from the hypothyroid animals consisted of <1% alkali-stable fibres as compared to 16% for the euthyroid muscles (Table 1). Whereas the hyperthyroid muscles were comprised of more than twice the percentage of alkali-stable fibres observed in the euthyroid muscles. Muscles of each group contained fibres with an intermediately intense M-ATPase stain. These fibres were most prevalent in the hyperthyroid muscles. They were classified as alkali-stable fibres in the present study.

The pH profiles of myosin ATPase activity are shown in

Fig. 1 Transverse sections from the soleus muscle of a, euthyroid b, hypothyroid and c, hyperthyroid rats stained for myofibrillar ATPase (×22).

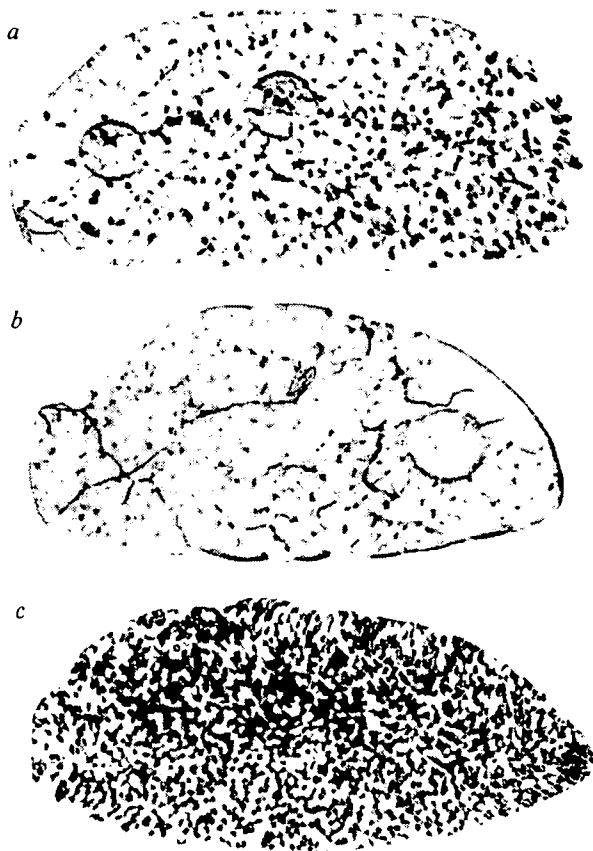


Fig. 2. The greatest differential effect on enzyme activity between the experimental groups was observed at pH 10.30. At this alkaline pH the euthyroid soleus exhibited only 54% of its activity at pH 7.40 as compared to 12% for the hypothyroid muscles and 236% for the hyperthyroid muscles. When comparing the activities at pH 10.30, the hypothyroid muscles had only 27% the activity of the euthyroid muscles. Whereas the hyperthyroid had a fivefold greater activity than the euthyroid muscles and 20-fold more activity than the soleus muscles of the hypothyroid animals at that alkaline pH.

As in the histochemical procedure, the basis for resolving fast-twitch and slow-twitch myosin ATPase is its property of being alkali resistant and susceptible, respectively<sup>10</sup>. The change in the alkaline stability of myosin isolated from hypothyroid and hyperthyroid muscles clearly shows that this property of the myosin protein has been transformed and that the transformation occurs in both directions, depending on the thyroid state. These biochemical findings, besides quantifying the changes in ATPase activity, have confirmed the histochemical observations for these experimental conditions. This is important since in certain conditions the histochemical method of staining for M-ATPase can be misleading<sup>14,15</sup>. Further evidence for the validity of the M-ATPase stain was a complete reversal of the staining intensity of fast and slow fibres at pH 4.35, as occurs in normal muscle. The close agreement between the percentage increase in alkali-stable fibres (19%) and myosin ATPase activity (39%) at pH 7.40, considering fast-twitch myosin has three times the specific catalytic activity of slow-twitch myosin, imparts further credibility to the M-ATPase stain. This comparison, however, is not as highly correlated for the hypothyroid muscle.

It is well documented that fast-twitch and slow-twitch muscle myosin are qualitatively distinct proteins<sup>10,11,16</sup>. It is widely accepted that these differences in myosin are primarily regulated by neural factors<sup>9,11,16,17</sup>. Our results indicate that the thyroid state of an animal also has the potential for determining the phenotype of skeletal muscle myosin. The extent of the changes in myosin ATPase activity in soleus muscles which had been hyperthyroid for only six weeks was similar to that of changes observed in soleus muscle following long-term (6–15 months) cross-innervation with a fast nerve<sup>11,16,17</sup>. Bárány and Close<sup>11</sup> observed a 50% increase in soleus myosin ATPase at pH 7.40 following 10–15 months of cross-innervation with a nerve normally innervating a fast-twitch muscle, as compared to a 39% increase for the hyperthyroid soleus. At pH 10.0, cross-innervated soleus muscle had a 125% increase in ATPase activity as compared to 70% for the hyperthyroid soleus at

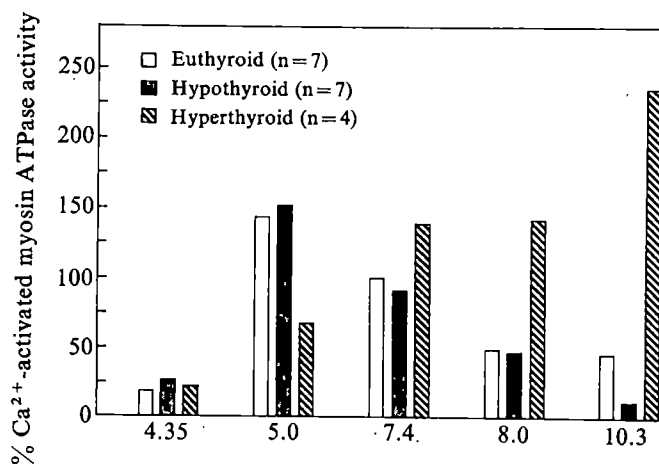


Fig. 2 % of Ca<sup>2+</sup>-activated myosin ATPase activity relative to 100% for the euthyroid muscle at pH 7.40. The euthyroid soleus muscle at pH 7.40 at 37 °C had an activity of 0.18  $\mu$ mol per mg myosin protein per min.

pH 10.30. In other studies<sup>16,17</sup> the soleus exhibited increases of 35–75% in myosin ATPase (pH 7.8–8.0) 6–12 months following cross-innervation, whereas the hyperthyroid soleus had 186% greater activity than the euthyroid at pH 8.00. Furthermore, the extent of this transformation is probably not fully manifest in this six-week time period in the hyperthyroid muscle but it is probably nearly complete in the long-term cross-innervation studies. Longer term studies are in progress to allow for the changes to become more fully established, along with the added purpose of further categorising the changes in the myosin protein.

The findings of this investigation offer little insight into the mechanism by which the thyroid state alters the character of skeletal muscle myosin. It is possible the thyroidal influence directly and selectively stimulated muscle gene expression, affected neural trophic regulation, or produced other hormonal imbalances which induced this transformation.

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- Goodkind, M. J., Dambach, G. E., Thyrum, P. T. & Luchi, R. J. *Am. J. Physiol.* 226, 66–72 (1974).
- Rovetto, M. J., Hjalmarson, A. C., Morgan, H. E., Barrett, M. J. & Goldstein, R. A. *Circ. Res.* 31, 397–409 (1972).
- Thyrum, P. T., Kritcher, E. M. & Luchi, R. J. *Biochem. biophys. Acta* 197, 335–336 (1970).
- Yazaki, Y. & Raben, M. S. *Circ. Res.* 36, 208–215 (1975).
- Kimata, S. & Morkin, E. *Am. J. Physiol.* 221, 1706–1713 (1971).
- Kubišta, V., Kubištová, J. & Pette, D. *Eur. J. Biochem.* 18, 553–560 (1971).
- Cooperstein, S. J., Lazarow, A. & Kurfess, N. J. *J. biol. Chem.* 186, 129–139 (1950).
- Padykula, H. A. & Herman, F. J. *Histochem. Cytochem.* 3, 170–195 (1955).
- Guth, L. & Samaha, F. J. *Expl Neurol.* 25, 138–152 (1969).
- Samaha, F. J., Guth, L. & Albers, R. W. *J. biol. Chem.* 245, 219–224 (1970).
- Bárány, M. & Close, R. I. *J. Physiol., Lond.* 213, 455–474 (1971).
- Rockstein, M. & Herron, P. W. *Analyt. Chem.* 23, 1500–1501 (1951).
- Lowry, O. H., Rosebrough, H. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* 193, 265–275 (1951).
- Guth, L. & Samaha, F. J. *Expl Neurol.* 34, 465–475 (1972).
- Guth, L. *Expl Neurol.* 41, 440–450 (1973).
- Jean, D. H., Guth, L. & Albers, R. W. *Expl Neurol.* 38, 458–471 (1973).
- Buller, A. J., Mommaerts, W. F. H. M. & Seraydarian, K. *J. Physiol., Lond.* 205, 581–597 (1969).

## Hydrolysis of polyesters by lipases

INCREASING public concern about the treatment of waste materials has stimulated the study of the biodegradation of synthetic polymers. Among synthetic polymers, aliphatic polyesters are generally known to be susceptible to biological attack<sup>1–5</sup>, but there are few reports of enzymes involved in their degradation. Bell *et al.*<sup>6</sup> recently showed that the molecular weight of polycaprolactone (PCL) decreases on exposure to the acid protease from *Rhizopus chinensis* for 6–10 d (decreasing from 13,000 to 10,000). In addition, Tabushi *et al.*<sup>7</sup> have found that polyesters composed of phenyllactic acid and lactic acid are hydrolysed by  $\alpha$ -chymotrypsin. We showed previously that a polyester-degrading enzyme from *Penicillium* sp. strain 14-3, purified to a homogeneous state as exhibited by ultracentrifugal analysis and polyacrylamide gel electrophoresis, has properties resembling lipase<sup>8</sup>. It was not previously recognised that lipase acts on polyesters. We report here that several commercial lipases and an esterase also hydrolyse polyesters, and that *Rh. delemar* lipase is capable of hydrolysing various kinds of polyesters.

PCL and polypropiolactone were prepared by ring opening polymerisation of  $\epsilon$ -caprolactone<sup>9</sup> and  $\beta$ -propiolactone respectively in benzene in a nitrogen atmosphere at 60 °C with a diethylzinc–water catalyst system. Poly- $\beta$ -methylpropiolactone (poly- $\beta$ -hydroxybutyrate) was made from  $\beta$ -methylpropiolactone by the method of Yamashita *et al.*<sup>10</sup> with a triethylaluminium–water catalyst system. Other saturated aliphatic polyesters were synthesised by a melt polycondensation technique<sup>11</sup>, and unsaturated polyesters were synthesised by high temperature solution polycondensation<sup>12</sup>. All alicyclic and aromatic polyesters were from Nihon Chromat except poly (tetramethylene terephthalate), from Union Carbide. The number average molecular weight ( $\bar{M}_n$ ) was measured by the vapour pressure equilibrium method with a Hitachi 117 apparatus.

Fig. 1 Gas chromatograms of the hydrolysis products of PEA by *Rh. delemar* lipase. PEA powder was filtered from the reaction mixture which was then analysed (a). The concentrated and dried reaction mixture was then esterified with diazomethane in ether (b). The hydrolysate of (a) with alkali was also analysed (c). Chromatographic conditions: Shimadzu GC-5A (flame ionisation) with glass column (1 m  $\times$  0.3 cm internal diameter) packed with Tenax GC. Temperature programmed at 5° per min from 135° to 345 °C (dashed line). Flow rate of both nitrogen and hydrogen was 50 ml per min. Arrows indicate ethylene glycol (EG) and adipic acid (AA).

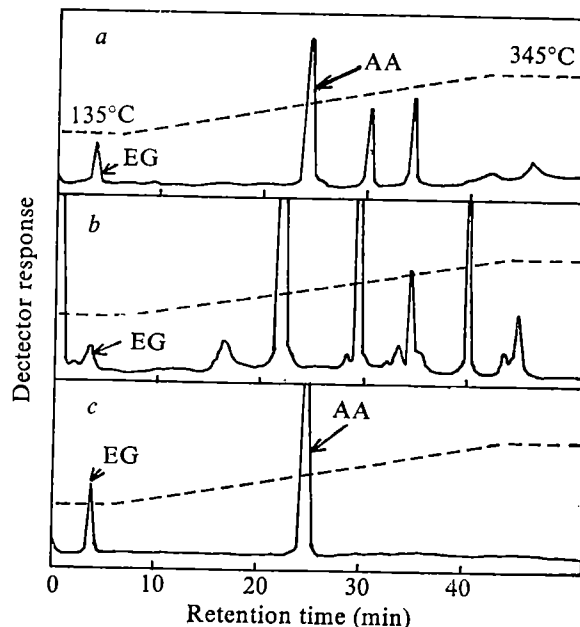


Table 1 Hydrolysis of polyesters by lipases

		TOC concentration (p.p.m.)			
Enzyme control		Formed by hydrolysis of polyester			
		Poly (ethylene adipate)	Poly-caprolactone	Poly(cyclohexylene-dimethyl succinate)	Poly(2,2-dimethyl-trimethylene isophthalate)
<i>Achromobacter</i> sp.	10	880	110	60	0
<i>Candida cylindracea</i>	5	3900	70	0	0
<i>Geotrichum candidum</i>	10	140	0	0	0
<i>Rhizopus arrhizus</i>	10	7400	60	40	0
<i>Rhizopus delemar</i>	5	6100	110	70	0
Hog liver (esterase)	30	940	90	120	0
(Substrate control)	—	(330)	(150)	(70)	(20)

Each reaction mixture contained 400  $\mu$ mol of phosphate buffer (pH 7.0), 300 mg of the polyester powder (particle size less than about 1 mm) and enzyme in a total volume of 10 ml. The enzyme concentration in each reaction mixture was adjusted to a degree of hydrolytic activity capable of liberating 420  $\mu$ mol of fatty acid from olive oil per 60 min at 37 °C at pH 7.0 (see ref. 8). Reaction mixtures were incubated on a rotary shaker at 180 r.p.m. at 30 °C for 16 h. After incubation, the TOC concentration was measured. No effect of the interaction between each substrate and inactivated enzyme preparation was observed. For example, when *G. candidum*, *Rh. arrhizus* or *Rh. delemar* lipase which was treated with a boiling water for 10 min, was allowed to react with PEA (PCL), the TOC concentrations in the filtrate of the reaction mixtures were 340 (150), 340 (160) and 330 (150) p.p.m. respectively as compared with 330 (150) p.p.m. of substrate control. The effect could not be examined in hog liver esterase because it was insolubilised by the heat treatment.

*Achromobacter* sp. and *Candida cylindracea* lipases (Meito Sangyo) were purified by gel filtration on Sephadex G-100 (2.6  $\times$  79 cm) from crude preparations. Ultracentrifugally homogeneous preparations of *Geotrichum candidum* and *Rh. delemar* lipases were from Seikagaku Kogyo and partially purified preparations of *Rh. arrhizus* lipase and hog liver esterase were from Boehringer Mannheim Yamanouchi.

Hydrolysis of polyesters was measured by the rate of their solubilisation, and the measurement process does not necessarily involve complete hydrolysis into the constituent parts. The rate was determined by measuring the water-soluble total organic carbon (TOC) concentration at 30 °C in the reaction mixture using a Toshiba-Beckman TOC analyser. The precise reaction rates of polyester hydrolysis cannot be determined by this method, however, since the polyester powder particles in the reaction mixture vary in size and the solubility of the oligomers

formed by polyester hydrolysis differs depending on the kind of polyester. In the substrate and enzyme controls, enzyme or substrate was omitted from the reaction mixture.

Aliphatic polyesters, poly(ethylene adipate) (PEA) and PCL were hydrolysed by lipases from various microorganisms and hog liver esterase with the results shown in Table 1. *Rh. arrhizus* and *Rh. delemar* lipases showed especially strong activity. The action of hog liver esterase on PEA produced much more adipic acid and ethylene glycol, constituents of PEA, than did lipases. This suggests that the biodegradable property of aliphatic polyester comes from its susceptibility to hydrolysis by lipases or esterases. An alicyclic polyester, poly(cyclohexylenedimethyl succinate), was also hydrolysed by lipases from *Achromobacter* sp., *Rh. arrhizus* and *Rh. delemar* and by hog liver esterase. Of these, hog liver esterase showed the strongest activity. In contrast, an aromatic polyester, poly(2,2-dimethyl-

Table 2 Hydrolysis of polyesters by *Rh. delemar* lipase

Polyester	Mn	Powder size	TOC concentration (p.p.m.)	
			Substrate control	Formed by lipase
Poly(ethylene adipate)	2,720	C	330	8,360
Poly(ethylene suberate)	4,050	B	250	1,020
Poly(ethylene azelate)	4,510	B	570	3,080
Poly(ethylene sebacate)	1,570	A	390	550
Poly(ethylene decamethylate)	1,610	B	100	180
Poly(tetramethylene succinate)	4,240	A	270	150
Poly(tetramethylene adipate)	1,790	B	520	3,360
Poly(tetramethylene sebacate)	2,440	A	130	980
Poly(hexamethylene sebacate)	5,820	B	40	380
Poly(2,2-dimethyltrimethylene succinate)	2,370	A	360	240
Polypropiolactone	4,270	A	580	2,240
Poly-DL- $\beta$ -methylpropiolactone	8,190	E	250	10
Polycaprolactone	6,740	A	150	310
Poly( <i>cis</i> -2-butene adipate)	2,700	C	1,270	580
Poly( <i>cis</i> -2-butene sebacate)	6,190	B	130	300
Poly( <i>trans</i> -2-butene sebacate)	3,560	A	910	340
Poly(2-butyne sebacate)	4,930	C	210	670
Poly(hexamethylene fumarate)		A	60	35
Poly( <i>cis</i> -2-butene fumarate)		B	390	30
Poly(tetramethylcyclobutane succinate)	3,440	E	10	0
Poly(cyclohexylenedimethyl succinate)	3,910	B	70	130
Poly(cyclohexylenedimethyl adipate)	3,250	B	80	200
Poly(tetramethylene terephthalate)		C	0	0
Poly(ethylene tetrachlorophthalate)	1,670	A	0	0
Poly(2,2-dimethyltrimethylene isophthalate)		A	20	0

All polyesters but two were prepared into powders at 30 °C by grinding. The exceptions were poly(tetramethylene terephthalate) which was powdered with a centrifugal ball mill and polycaprolactone which was powdered from a solution in chloroform by a precipitation method. The particle size of each polyester powder was ranked A, B, C, D or E, corresponding respectively to roughly less than 0.25 mm, less than 0.50 mm, less than 1.0 mm, 0.25–1.5 mm, 0.25–3 mm. Each reaction mixture contained 400  $\mu$ mol of phosphate buffer (pH 6.0), 300 mg of the polyester powder and 0.3 mg of *Rh. delemar* lipase in a total volume of 10.0 ml. The reaction mixtures were incubated in the same way as Table 1. The TOC concentration of the enzyme control was 10 p.p.m.



trimethylene isophthalate), was not hydrolysed by any of the enzyme preparations.

*Rh. delemar* lipase purified to homogeneity in the ultracentrifuge was found capable of hydrolysing various kinds of polyesters (Table 2). PEA was the best substrate tested, and activity was relatively high with poly(ethylene azelate). Polypropiolactone and PCL were degraded, but not poly(DL-β-methylpropiolactone), the D-isomer of which is known to be one of the materials stored by bacteria and algae. Unsaturated polyesters from 2-butenediol were also degraded, but polyesters with even a few hybrid bonds among the polymer chains, such as those induced from poly(hexamethylene fumarate) and poly(cis-2-butene fumarate), were hardly degraded. Two alicyclic polyesters, poly(cyclohexylenedimethyl succinate) and poly(cyclohexylenedimethyl adipate), were degraded fairly extensively. Aromatic polyesters such as poly(tetramethylene terephthalate), poly(ethylene tetrachlorophthalate) and poly(2,2-dimethyltrimethylene isophthalate) were not degraded.

Amongst the products of PEA hydrolysis by *Rh. delemar* lipase, we detected not only the constituent parts of PEA—adipic acid and ethylene glycol—but also large quantities of PEA oligomers (Fig. 1). This may indicate that *Rh. delemar* lipase randomly splits ester bonds.

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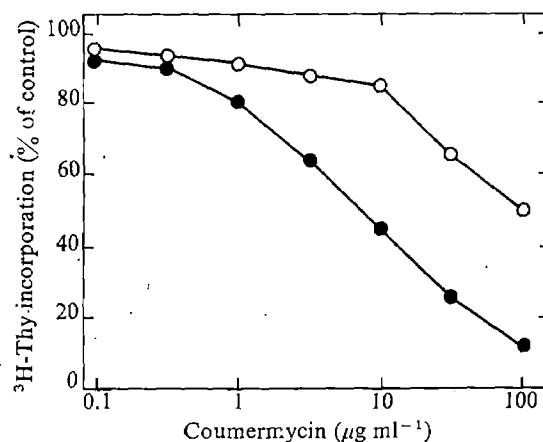
1. Darby, R. T. & Kaplan, A. M. *Appl. Microbiol.* **16**, 900–905 (1968).
2. Potts, J. E., Clendinning, R. A., Ackart, W. B. & Niegisch, W. D. *Am. chem. Soc., Polymer Preprints* **13**, 629–632 (1972).
3. Fields, R. D., Rodriguez, F. & Finn, R. K. *J. appl. Polym. Sci.* **18**, 3571–3579 (1974).
4. Diamond, M. J., Freedman, B. & Garibaldi, J. A. *Int. Biodetn Bull.* **11**, 127–132 (1975).
5. Tokiwa, Y., Ando, T. & Suzuki, T. *J. Ferment. Technol.* **54**, 603–608 (1976).
6. Bell, J. P., Huang, S. J. & Knox, J. R. *U. S. NTIS, AD-A Rep. No. 009577* (1974).
7. Tabushi, I., Yamada, H., Matsuzaki, H. & Furukawa, J. *J. Polym. Sci. Polym. Lett. Ed.* **13**, 447–450 (1975).
8. Tokiwa, Y. & Suzuki, T. *Agric. Biol. Chem.* **41**, 265–274 (1977).
9. Lunberg, R. D., Koleske, J. V. & Wischmann, K. B. *J. Polym. Sci. A-1*, **7**, 2915–2930 (1969).
10. Yamashita, Y., Tuda, T., Ishikawa, Y. & Miura, S. *J. chem. Soc. Japan, Ind. Chem. Sec.* **66**, 110–115 (1963).
11. Carothers, W. H. & Arvin, J. A. *J. Am. chem. Soc.* **51**, 2560–2570 (1929).
12. Batzer, H., Holtschmidt, H., Wiloth, F. & Mohr, B. *Makromol. Chem.* **7**, 82–103 (1951).

## Involvement of DNA gyrase in bacteriophage T7 DNA replication

NOVOBIOCIN and the related antibiotic coumermycin A<sub>1</sub> (referred to as coumermycin) inhibit the supercoiling of circular double-stranded DNA catalysed by *Escherichia coli* DNA gyrase<sup>1</sup>. They also inhibit the replication of chromosomal DNA in *E. coli* cells<sup>2</sup>, and of circular double-stranded DNA in cell-free systems from *E. coli*<sup>3–6</sup>. The activity of DNA gyrase obtained from a coumermycin-resistant (*cou<sup>r</sup>*) mutant and the replication of colicin E1 plasmid (*Col E1*) DNA in a cell extract of the mutant are resistant to both drugs<sup>7</sup>. These results show that DNA gyrase is an essential component of these systems for the replication of the circular double-stranded DNA. It is interesting to examine whether DNA gyrase also has a role in replication of linear double-stranded DNA. The DNA of *E. coli* bacteriophage T7 is linear and double stranded<sup>8</sup>. Linear replicating molecules have been isolated from infected cells and it has been claimed that the phage DNA replicates as a linear form, at least in the first round of replication<sup>9</sup>. We show here that the replication of phage T7 DNA, including the first round, is inhibited by coumermycin.

The effect of various concentrations of coumermycin on DNA synthesis in coumermycin-sensitive and resistant bacteria during 30 min after infection of phage T7 was examined. T7 DNA synthesis was inhibited by coumermycin, to a greater extent in the sensitive bacteria than in the resistant bacteria (Fig. 1). To attain a similar extent of

inhibition, an approximately 10 times higher concentration of the drug was needed for the resistant bacteria than for the sensitive bacteria. At a given concentration of the drug, T7 DNA synthesis was inhibited to about the same extent as chromosomal DNA synthesis in the uninfected cells. This was true of both the sensitive and the resistant bacteria. On the other hand, it has been shown that a 50-fold lower concentration of the drug is sufficient to produce comparable inhibition in toluene-treated cells (refs 4, 13 and unpublished results). Thus the T7-infected cells seem to retain the same limited permeability to the drug as the uninfected cells. Phage DNA synthesis (Fig. 1) and chromosomal DNA synthesis (data not shown) in the resistant bacteria were inhibited by high concentrations of coumermycin. It is known that high concentrations (25 μg ml<sup>-1</sup> or higher) of coumermycin significantly inhibit DNA gyrase purified from the resistant bacteria (M. Gellert, personal communication) as well as other enzymes, such as DNA polymerase III and DNA-dependent RNA polymerase of *E. coli*<sup>14</sup>. The resistant strain used in this experiment was selected as a spontaneous drug-resistant mutant and its mutation was mapped close to the *dnaA* locus by P1-mediated transduction. Moreover, when *bglC<sup>+</sup>* (the *bgl* locus is known to be located near the *dnaA* locus<sup>14</sup>) transductants of the resistant strain were selected, about 20% of them were found to be simultaneously coumermycin-sensitive, and two representative drug-sensitive transductants showed a drug-sensitivity of T7 growth similar to that of the



**Fig. 1** Effect of coumermycin on DNA synthesis in T7-infected cells. NI746 (●), a low thymine-requiring mutant of NT525 *Su-bglC<sup>+</sup>cou<sup>r</sup>* and its spontaneous coumermycin-resistant (*cou<sup>r</sup>*) derivative, NI751 (○) were used. The *cou<sup>r</sup>* mutation of NI751 was mapped close to the *dnaA* locus. DNA synthesis in toluene-treated cells of NI751 and *Col E1* DNA replication in an extract of this strain were resistant to coumermycin. Cells were grown to  $3 \times 10^8$  per ml in OC medium (a minimal medium with casamino acids (Difco))<sup>10</sup> supplemented with thymine 2 μg ml<sup>-1</sup> at 37°C, centrifuged and suspended in one-tenth volume of the adsorption buffer (10mM Tris-HCl, pH7, 1mM MgCl<sub>2</sub>, 0.5% NaCl, 10 μg ml<sup>-1</sup> gelatin, 0.4% casamino acids) with various concentrations of coumermycin (from J. Davies, University of Wisconsin). Coumermycin was dissolved in dimethyl sulphoxide and an equal amount of the solvent was added to the control cultures. Bacteria were infected with phage T7 at a multiplicity of five by incubating for 7 min at 30°C. The adsorption mixtures were diluted 10-fold in OC medium with <sup>3</sup>H-thymine (1.2 Ci mmol<sup>-1</sup>, NEN) and various concentrations of the drug. After incubating at 30°C for 30 min, acid-insoluble radioactivity in 0.1 ml of each culture was determined<sup>10</sup>—100% corresponds to about 12,000 c.p.m. A DNA-DNA hybridisation experiment<sup>11</sup> was performed with the samples made from the infected sensitive and resistant bacteria incubated with or without 100 μg ml<sup>-1</sup> coumermycin. In the sample made from the sensitive bacteria without the drug and those from the resistant bacteria with or without the drug, more than 80% of the labelled material was phage T7 DNA, whereas 75% was bacterial DNA in the sample made from the sensitive bacteria with the drug (data not shown). It is known that bacterial DNA synthesis does not stop immediately by T7-infection<sup>12</sup>, and it is also known that bacterial DNA synthesis does not stop immediately after addition of coumermycin<sup>2</sup>.

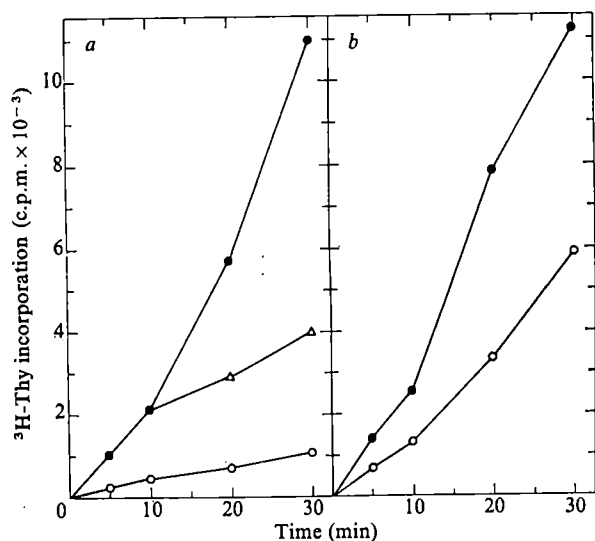


Fig. 2 Kinetics of DNA synthesis by T7-infected cells of NI746-*cou*<sup>+</sup> (a) and NI751-*cou*<sup>+</sup> (b). Methods used were generally as described in Fig. 1. Cells were grown in OC medium supplemented with thymine ( $2 \mu\text{g ml}^{-1}$ ), centrifuged and suspended in the adsorption buffer with (○) or without (●) coumermycin ( $100 \mu\text{g ml}^{-1}$ ). The cells were then infected with phage T7 at a multiplicity of five. After incubation for 7 min at  $30^\circ\text{C}$ , mixtures were diluted in the same medium with  $^3\text{H}$ -thymine ( $1.2 \text{ Ci mmol}^{-1}$ ) and incubated at  $30^\circ\text{C}$ . After incubation for 10 min, the culture of the infected sensitive bacteria without the drug was divided into two portions, to one of which coumermycin ( $100 \mu\text{g ml}^{-1}$ ) was added and incubation was continued (△). At intervals aliquots (0.1 ml) were withdrawn and acid-insoluble radioactivity was determined.

sensitive strain used in this experiment. Therefore, the possibility that the observed resistance of T7 growth to the drug was due to some unidentified drug-resistant mutant enzyme which might be involved in T7 growth should be very scarce. The differential effect of the drug on T7 DNA synthesis in the sensitive and the resistant bacteria indicates that the inhibition of the activity of DNA gyrase by coumermycin caused the inhibition of T7 DNA synthesis in the sensitive bacteria.

DNA gyrase could act directly on T7 DNA molecules, or it could act on the bacterial chromosome to produce and maintain its negatively supercoiled structure, which might be necessary for T7 DNA replication. When coumermycin  $100 \mu\text{g ml}^{-1}$  was added at the beginning of infection, DNA synthesis in the infected sensitive bacteria was severely inhibited (Fig 2a), whereas that in the resistant bacteria was relatively less inhibited (Fig. 2b). A similar inhibition of DNA synthesis in the infected sensitive bacteria was observed when the drug was added 10 min after infection (Fig 2a). Since the bacterial chromosomes are extensively broken down by that time<sup>15</sup>, the inhibition of T7 DNA synthesis is not likely to be an indirect effect due to action of the drug on the bacterial chromosomes. This in turn shows that DNA gyrase acts on T7 DNA molecules.

To determine whether even the first round of replication of T7 DNA molecules is inhibited by coumermycin, the fate of the parental DNA after infection was examined. In this experiment, T7am233 (gene 6) was used with a multiplicity of infection of less than one to eliminate possible complexities in the analysis due to recombination and concatemer formation. It is known that neither recombination nor formation of concatemers of T7 DNA occurs in the absence of a functional gene 6 product, although several rounds of semiconservative DNA replication take place<sup>16-18</sup>. Both the sensitive and resistant bacteria incubated for 20 min in 5-bromodeoxyuridine (BrdUrd)-supplemented medium were infected with  $^{32}\text{P}$ -labelled T7am233 and incubated for 12.5 min at  $30^\circ\text{C}$  with or without  $100 \mu\text{g ml}^{-1}$

coumermycin. DNA was extracted and analysed by equilibrium CsCl density gradient centrifugation. For the DNA prepared from the infected sensitive bacteria incubated without the drug, most of the labelled DNA appeared at the position of fully light (LL) DNA, and about 25% formed a broad band with a peak approximately at the position of half-heavy (HL) DNA (Fig. 3a). The shoulder on the heavier side of HL DNA probably consists of replicative intermediates in the second or later round of replication. T7am233 DNA replication in each infected cell was quite heterogeneous. The DNA replication of T7am<sup>+</sup> as well as a gene 6 amber mutant has been shown to be rather heterogeneous in the experiments similar to that shown here; a considerable portion of the parental DNA still forms a band at the position of LL DNA, even when the progeny DNA labelled with BrdUrd appears at the position of fully heavy (HH) DNA, and replicated DNA molecules form rather broad bands at the positions of HL and HH DNA<sup>16,17</sup>. For the DNA prepared from the infected sensitive bacteria incubated with coumermycin, however, practically no labelled DNA appeared in the position of HL DNA (Fig. 3c). In contrast, when the resistant bacteria were used, the amount of labelled DNA

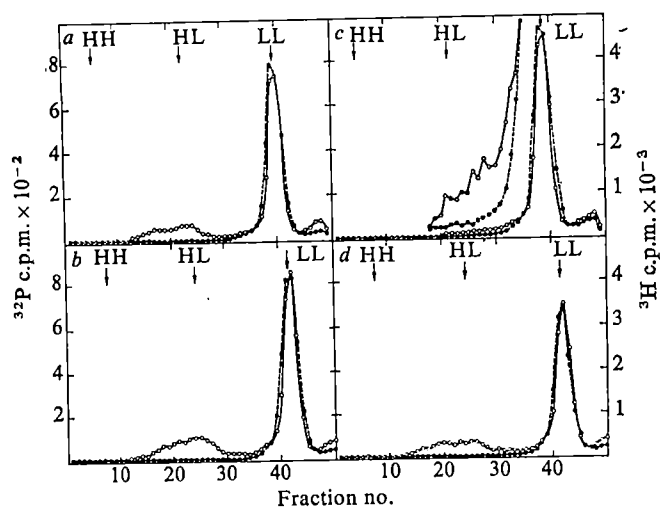


Fig. 3 CsCl density gradient centrifugation of DNA extracted from cells of NI746-*cou*<sup>+</sup> (a and c) and NI751-*cou*<sup>+</sup> (b and d) infected with  $^{32}\text{P}$ -labelled T7am233. Cells were grown in OC medium supplemented with thymine ( $2 \mu\text{g ml}^{-1}$ ), centrifuged and suspended in OC medium supplemented with BrdUrd ( $5 \mu\text{g ml}^{-1}$ , Sigma) in place of thymine and further incubated for 20 min at  $37^\circ\text{C}$  to replace the intracellular pool of nucleotides. Cells were then centrifuged, suspended in the adsorption buffer with (c and d) or without (a and b) coumermycin ( $100 \mu\text{g ml}^{-1}$ ), and infected with  $^{32}\text{P}$ -labelled ( $40 \text{ mCi mmol}^{-1}$ ) T7am233 (phage strain obtained from H. Ogawa, University of Osaka) at a multiplicity of 0.6. After incubation for 5 min at  $30^\circ\text{C}$ , the cultures were diluted in OC medium supplemented with BrdUrd and with or without the drug, and incubated at  $30^\circ\text{C}$ . At 12.5 min after the dilution, the reaction was stopped by addition of four volumes of cold SSC ( $0.15 \text{ M NaCl}$ ,  $0.015 \text{ M Na}_2\text{ citrate}$ )- $5 \text{ mM KCN}$ . Cells were centrifuged and suspended in  $0.2 \text{ ml}$  of SSC (pH adjusted to 8.0)- $10 \text{ mM EDTA}$ - $10 \text{ mM KCN}$ . Lysozyme (Sigma) was added at a concentration of  $500 \mu\text{g ml}^{-1}$  and the mixtures were frozen and thawed three times. After incubation for 15 min at  $37^\circ\text{C}$ , sodium *N*-lauroyl sarcosinate (Sigma) was added at a concentration of  $0.1\%$  and the mixtures were incubated for 15 min at  $65^\circ\text{C}$ . The cell lysates were then treated with proteinase K ( $200 \mu\text{g ml}^{-1}$ , E. Merck) at  $37^\circ\text{C}$  for 2 h. The lysates were diluted with SSC and after addition of  $^3\text{H}$ -labelled ( $2.5 \times 10^4 \text{ c.p.m.}$ ) *E. coli* DNA, brought to a final volume of  $7.5 \text{ ml}$  with a density of  $1.73 \text{ g ml}^{-1}$  by adding CsCl. They were centrifuged in a Beckman 40 rotor for 50 h at  $36,000 \text{ r.p.m.}$  at about  $15^\circ\text{C}$ . Each gradient was collected from the bottom of the tube to give about 50 fractions, and  $^{32}\text{P}$  (○) and  $^3\text{H}$  (●) counts were determined. Approximately 80% of the input radioactivity was recovered from each gradient. Positions for light (LL), half-heavy (HL) and heavy (HH) DNA are indicated by the arrows. In c, the distribution of  $^{32}\text{P}$  and  $^3\text{H}$  counts in the fractions 18-35 is also shown on a 10-fold expanded scale.

which formed a band around the position of HL DNA was only slightly reduced by the addition of the drug (Fig. 3b, d). These results indicate that the inhibition of replication of T7am233 DNA molecules in the sensitive bacteria was due to inhibition of DNA gyrase by coumermycin.

The inhibition of the first round of replication could occur at initiation and/or at an early stage of DNA replication. In the preparation made from the infected sensitive bacteria incubated with the drug, a small but significant amount of labelled DNA was found between the positions of HL DNA and LL DNA (see Fig. 3c inset), even though practically no band of the labelled DNA was found in the positions of the HL or heavier DNA. These molecules are likely to be replicative intermediates arrested by the drug early in replication, rather than those on the way of the normal replication resulting from the incomplete inhibition by the drug of the initiation of DNA replication. This leads to the conclusion that DNA gyrase is involved at least in the stage of elongation of DNA chains.

Phage T7 DNA has redundant ends and could circularise through that structure<sup>19</sup>. Circular molecules have been only rarely observed in T7am<sup>+</sup> DNA obtained from bacteria at an early stage of infection, however (refs 2, 9 and unpublished results of T. Ogawa and J.T.). Use of T7am233 reduced the possibility of circularisation of phage DNA molecules mediated by the product of gene 6. Because the DNA of T7am<sup>+</sup> as well as a gene 6 amber mutant<sup>17</sup> seems to replicate as a linear molecule, the inhibition of the first round of replication of T7am233 DNA molecules by coumermycin implies that DNA gyrase is involved in the replication of linear double-stranded DNA.

When a duplex DNA molecule replicates, the parental strands must unwind to serve as templates. If the replicating molecule is circular and covalently closed, at least a temporary nicking of one of the strands is essential for the unwinding. On the other hand, if the replicating molecule is linear, the unwinding could be accomplished by relative rotation of the replicated region in relation to the unreplicated region of the molecule without nicking of the strands. But, such rotation for unwinding of a long duplex DNA molecule *in vivo* is quite improbable, if one considers the necessity of simultaneous rotation of molecules attached to the DNA molecule, such as the machinery for transcription and translation and cellular structures like membranes<sup>20</sup>. It has been suggested that phage T7 DNA in infected cells is associated with the bacterial membrane<sup>21-23</sup>. The unwinding could be facilitated by nicking and closing of one strand which would occur at or a little ahead of the replicating point. A nicking and closing enzyme may act to relieve the positive supercoiling strain imposed by replication, or more actively, to facilitate separation of the parental strands by imposing a negative supercoiling strain. DNA gyrase would be a suitable enzyme to act in this process.

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- Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, H. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3872-3876 (1976).
- Ryan, M. J. *Biochemistry* **15**, 3769-3777 (1976).
- Staudenbauer, W. L. *J. molec. Biol.* **96**, 201-205 (1975).
- Ryan, M. J. & Wells, R. D. *Biochemistry* **15**, 3778-3782 (1976).
- Staudenbauer, W. L. *Molec. gen. Genet.* **145**, 273-280 (1976).
- Sumida-Yasumoto, C., Yudelevich, A. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1887-1891 (1976).
- Gellert, M., O'Dea, M. H., Itoh, T. & Tomizawa, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4474-4478 (1976).
- Studier, F. W. *J. molec. Biol.* **11**, 373-390 (1965).

- Dressler, D., Wolfson, J. & Magazin, M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 998-1002 (1972).
- Sakakibara, Y. & Tomizawa, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 802-806 (1974).
- Denhardt, D. *Biochem. biophys. Res. Commun.* **23**, 641-646 (1966).
- Kelly, T. J., Jr & Thomas, C. A., Jr *J. molec. Biol.* **44**, 459-475 (1969).
- Moses, R. E. & Richardson, C. C. *Proc. natn. Acad. Sci. U.S.A.* **67**, 674-681 (1970).
- Bachman, B. J., Low, K. B., Taylor, A. L. *Bact. Rev.* **40**, 116-167 (1976).
- Sadowski, P. D. & Kerr, C. J. *J. Virol.* **6**, 149-155 (1970).
- Strätling, W., Krause, E. & Knippers, R. *Virology* **51**, 109-119 (1973).
- Lee, M. & Miller, R. C., Jr *J. Virol.* **14**, 1040-1048 (1974).
- Fröhlich, B., Powling, A. & Knippers, R. *Virology* **65**, 455-468 (1975).
- Ritchie, D., Thomas, C., MacHattie, L. & Wensink, P. *J. molec. Biol.* **23**, 365-376 (1967).
- Tomizawa, J. & Owaga, T. *Cold Spring Harb. Symp. quant. Biol.* **33**, 533-551 (1968).
- Strätling, W., Ferdinand, F. J., Krause, E. & Knippers, R. *Eur. J. Biochem.* **38**, 160-169 (1973).
- Pacumbaba, R. P. & Center, M. S. *J. Virol.* **12**, 855-861 (1973).
- Helland, D. & Nygaard, A. P. *FEBS Lett.* **50**, 13-16 (1975).

## Is the centriole bound to the nuclear membrane?

THE persistence of the centriole during interphase near the nucleus within the so-called cell centre seems to be a general feature of most animal cells. An association between the nucleus and the cell centre has been proposed and has been clearly shown in some cell types<sup>1,2</sup>. A permanent system involving chromosomes, spindle fibres and centrioles has been suggested by Lettre *et al.*<sup>3</sup>, and Aronson<sup>4</sup> has demonstrated the existence of a Colcemid-sensitive mobile system between the nucleus and a centre at the two-cell stage in the sea urchin *Lytechinus variegatus*. We show here that there is a physical association between the centriole and the nucleus in the rat liver cell which resists cold treatment, cell disruption and nuclear isolation. When nuclear envelopes are prepared from isolated nuclei, the association between the centriole and the nuclear envelope is maintained.

Nuclei from rat liver were prepared according to Blobel and Potter<sup>5</sup> with slight modifications to adapt the procedure to larger amounts of tissue. Liver tissue (33 g) was homogenised in 110 ml of 2.2 M sucrose-TKM (50 mM Tris-HCl pH 7.5, 25 mM KCl, 5 mM MgCl<sub>2</sub>) with a Chauveau type homogeniser (Plastimarne). The homogenate was filtered through 10 layers of cheese cloth and mixed with 220 ml of 2.2 M sucrose-TKM, using a glass rod. The suspension was transferred to cellulose nitrate tubes underlaid with 10 ml of 2.3 M sucrose-TKM and centrifuged for 1 h at 4 °C and 25,000 r.p.m. in an SW 25.2 rotor (Beckman). The nuclei were then washed by resuspension with 1 M sucrose-TKM and with 0.25 sucrose-TKM.

These nuclear preparations were pure as judged by electron microscopy. Nuclei showed intranuclear structures such as nucleoli and perinuclear and perinucleolar chromatin. The nuclear envelope was present, contained pores and had ribosomes associated with the outer leaflet. The chemical composition of these preparations was constant, suggesting good reproducibility of the isolation technique. The lipid phosphorus/DNA phosphorus ratio was  $0.060 \pm 0.002$  (over 20 determinations). This value is low, indicating a low cytoplasmic contamination. Presence of centrioles in such nuclear preparations was not detected by electron microscopy (although we did not make a systematic search using serial sections).

Nuclear envelopes were isolated from such nuclei by the use of heparin which, in suitable conditions, induces complete solubilisation of chromatin<sup>6,7</sup>. This allows the observation of well-preserved nuclear envelopes which have not been damaged by sonication, high salt or shearing through sucrose gradients. Nuclei were resuspended in 0.01 M Tris-HCl pH 8.0 containing 0.01 M Na<sub>2</sub>HPO<sub>4</sub> at a final concentration of 200 µg DNA per ml. Heparin was then added in an excess corresponding to 1.5 times (w/w) the amount of DNA present in the nuclear suspension. After short vortex mixing the nuclear suspension was diluted five times with buffer and nuclear membranes were sedimented by centrifugation for 40 min at 20,000 r.p.m. (45,000g). All operations were performed at 4 °C.

Electron microscopic observation of such nuclear envelopes showed a surprisingly high number of centrioles. Some examples

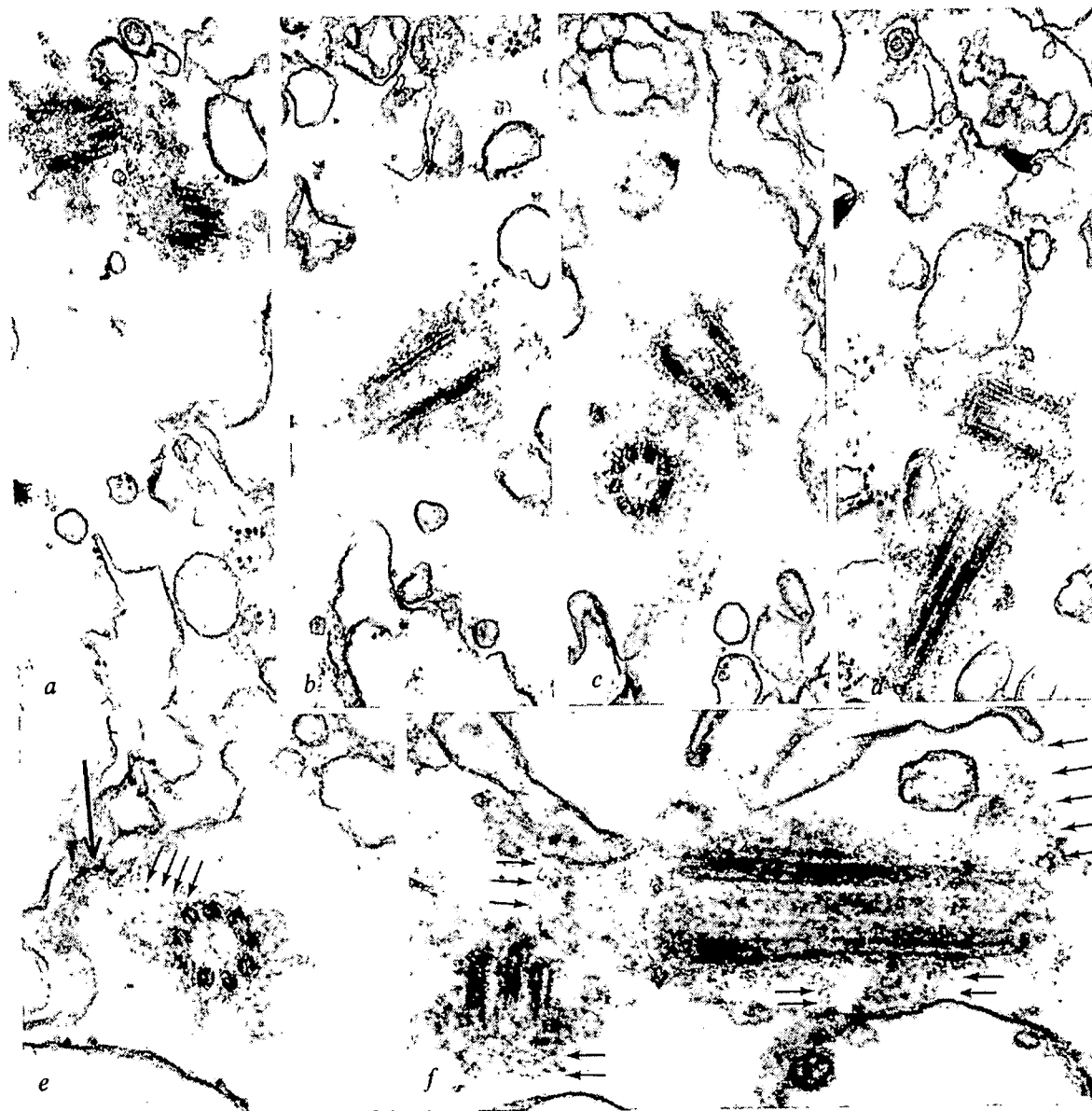


Fig. 1 Presence of centrioles in nuclear membrane pellets. Rat liver nuclei were resuspended at 4 °C in 0.01 M Tris-HCl pH 8.0 containing 0.01 M  $\text{Na}_2\text{HPO}_4$  and incubated with heparin ( $\text{Na}^+$  salt-grade I from Sigma) at a heparin to DNA ratio of 1.5 (w/w). After short vortexing, the nuclear suspension was diluted fivefold with buffer; nuclear membranes were sedimented immediately in that case at 45,000g for 40 min. Nuclear membrane pellets were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. They were postfixed in 2% osmium tetroxide, dehydrated in ethanol and embedded in Epon-Araldite. *a-d*, 42,000  $\times$ ; *e, f*, details showing apparent attachment (small arrows) of centrioles to membranes through dense material surrounding them. *e*, 60,000  $\times$ ; the large arrow indicates a nuclear pore. *f*, 72,000  $\times$ .

are shown in Fig. 1. They sometimes seemed to be bound to the nuclear envelope by the surrounding material (Fig. 1*e-f*). We assumed that the failure to see centrioles in preparations of whole isolated nuclei was due to the relative size of centrioles compared to nuclei. Such a difficulty should not occur with optical microscopy which allows the observation of whole nuclei. Isolated nuclei, suspended in the isolation buffer (0.05 M Tris-HCl, pH 7.5, 0.025 M KCl, 0.005 M  $\text{MgCl}_2$ ) showed irregular shapes, intranuclear organisation and nucleoli when observed by phase contrast microscopy. It remained difficult to detect the presence of centrioles in these nuclei.

The ability of low  $\text{Mg}^{2+}$  ( $5 \times 10^{-4}$  M) to decondense heterochromatin<sup>8,9</sup> was exploited to produce swollen and spherical nuclei with homogeneous nucleoplasm, in which all apparent organisation is lost except faint nucleoli. In such conditions, it was easy to detect centrioles associated with nuclei either by phase contrast microscopy or in polarised light. Several examples are given in Fig. 2. More than 50% of the nuclei in any pre-

paration had a centriole which could be recognised without ambiguity because of its typical aspect as two paired dots in phase contrast microscopy or polarised light microscopy. In rare circumstances, centrioles could be seen as two orthogonally oriented small bars (Fig. 2*f, j*); change in focus could show centrioles as black dots or refringent white dots (Fig. 2*e, i*). With some nuclei, by changing the focus, one could observe the centriole as two white dots, two black dots, a white and a black dot and the reverse situation (Fig. 2*e*). This is because of the complex spatial arrangement of centriole in two orthogonally oriented bars. In some cases varying the focus caused one or two other dense spots to appear on the same nucleus (Fig. 2*g, h, i*), but the meaning of this observation is not known.

In some nuclei, fibrillar structures could be seen originating in centriole. This would suggest the existence of an 'aster-like' system in the liver cell, and that part of it may remain associated with centriole during cell disruption and cold treatment.

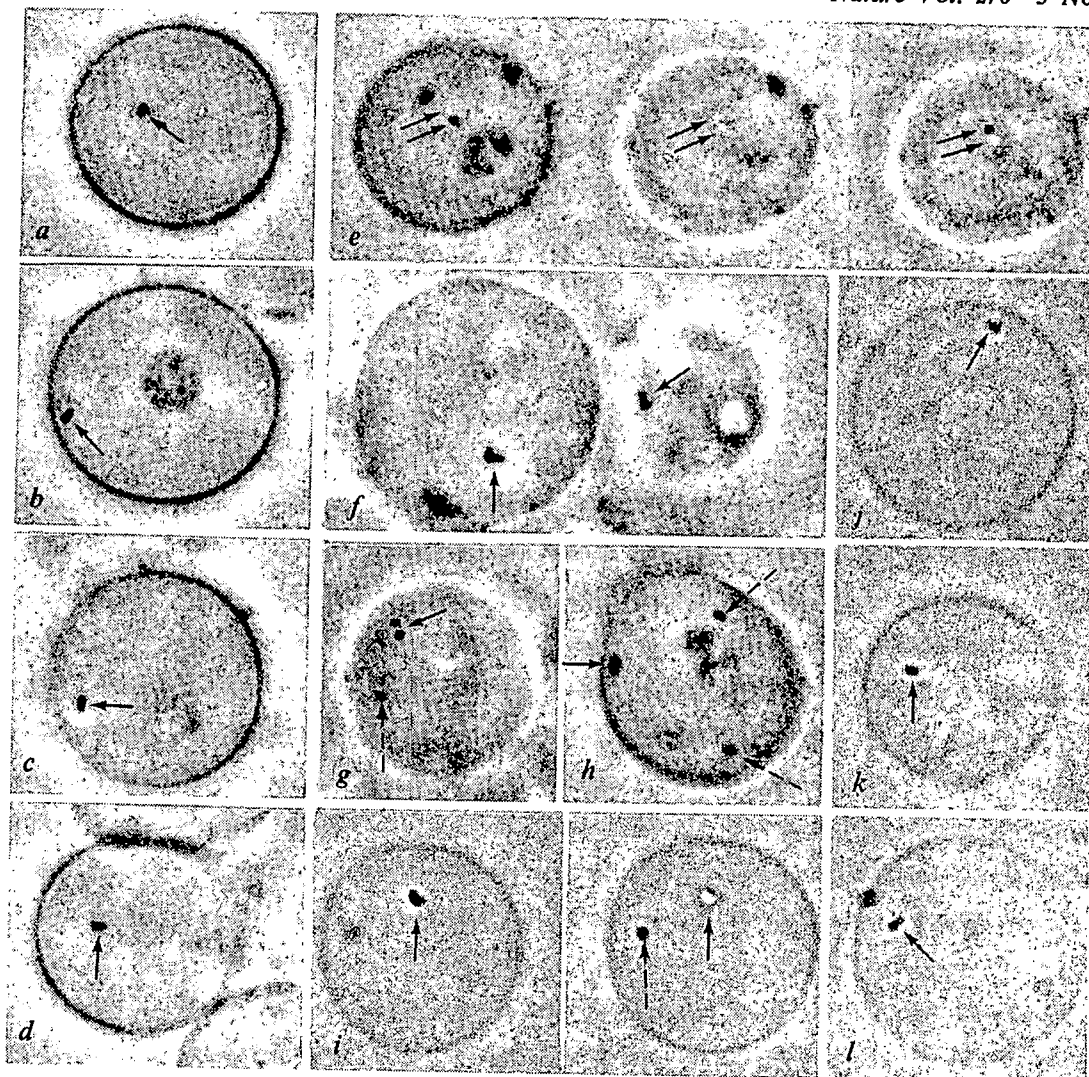


Fig. 2 Examples of rat liver nuclei resuspended at low magnesium concentration (0.05 M Tris-HCl pH 7.5, 0.025 M KCl,  $5 \times 10^{-4}$  M  $MgCl_2$ ) which show associated centrioles. Magnification is  $2,100 \times$ . Arrows indicate centrioles. *a-h*, Phase contrast microscopy; *i-l*, polarised light; *e* and *i*, various focusings on the same nucleus which show that centrioles can appear as two dark dots, two refringent dots or one dark and one refringent dot. *f* and *j*, centrioles appearing as two orthogonally-oriented bars. *g* and *h*, nuclei showing other dark dots (dotted arrows) beside centrioles.

This association centriole-nucleus is probably not restricted to the liver cell, as it was also seen in preparations of thymocyte nuclear membranes.

What is the significance of these observations? A major role has been attributed to the cytoskeleton in the control of cellular functions such as cell shape, cell movements, cell polarity and cell surface activity. This raises the question of the exact role of the association of the microtubule organising centre with centrioles during interphase.

One role for such a binding between nucleus and centriole could be the anchorage of nucleus into the cell cytoplasm. The methods introduced by Prescott *et al.*<sup>10,11</sup> to enucleate cells make use of cytochalasin B, and enucleated cytoplasts obtained in this way always contained centrioles<sup>12-14</sup>. One can therefore speculate that it is the disruption of the binding of nucleus to centriole which allows cell enucleation, although an alternative hypothesis has been proposed by Wright *et al.*<sup>15</sup> which makes the subplasmalemmal network of microfilaments the target of the cytochalasin B effect. The effect of cytochalasin B suggests that the binding of the nucleus to the centriole is at least in part realised by actin filaments. That not only actin is involved is indicated by the effect of colchicine<sup>1</sup> and colcemid<sup>2</sup> on the centriole-nucleus complex.

The well-known biological effect of cytochalasin B on cell cultures is to impair cytokinesis but not karyokinesis, leading to bi- or multi-nucleated cells<sup>15</sup>. Thus, the structural role proposed

here for the nucleus-centriole complex, that is, anchorage of the nucleus into the cell, would indeed have a functional counterpart.

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1. Bessis, M. & Breton-Gorius, J. *Nouvelle Rev. Française Hematologie* 7, 601-620 (1967).
2. Rondanelli, E. G., Carosi, G., Gerna, G. & Magliulo, E. *Acta Anat.* 70, 85-98 (1968).
3. Lettre, M. & Lettre, R. *Rev. Hemat.* 13, 337-362 (1958).
4. Aronson, J. F. J. *Cell Biol.* 51, 579-583 (1971).
5. Blobel, G. & Potter, V. R. *Science* 154, 1662-1665 (1966).
6. Bornens, M. *Nature* 244, 28-30 (1973).
7. Bornens, M. *Meth. Cell Biol.* 15, 163-175 (1977).
8. Daut-Mentire, P. J. *Microscopie* 3, 607-626 (1964).
9. Ollins, D. E. & Ollins, A. L. *J. Cell Biol.* 53, 715-736 (1972).
10. Prescott, D. M., Myerson, D. & Wallace, J. *Expl Cell Res.* 71, 480-485 (1972).
11. Prescott, D. M. & Kirkpatrick, J. B. *Meth. Cell Biol.* 7, 189-202 (1973).
12. Wise, G. E. & Prescott, D. M. *Expl Cell Res.* 81, 63-72 (1973).
13. Shay, J. W., Porter, K. R. & Prescott, D. M. *Proc. natn. Acad. Sci. U.S.A.* 71, 3059-3063 (1974).
14. Goldman, R. D., Pollack, R., Chang, C. M. & Bushnell, A. *Expl Cell Res.* 93, 175-183 (1975).
15. Wright, W. E. & Hayflick, L. *Proc. Soc. exp. Biol. Med.* 144, 587-592 (1973).
16. Carter, S. B. *Nature* 213, 261-264 (1967).



# reviews

## Islands in space

R. L. F. Boyd

*The High Frontier: Human Colonies in Space.* By G. K. O'Neill. Pp. 288. (Jonathan Cape: London, 1977.) £5.95.

A POPULATION of several thousand million people located in space colonies, within a mere 35 years of the initiation of a space colonisation programme, is typical of the thinking—one might even say the evangel—of Dr O'Neill. In this readable and extraordinarily optimistic promotion of the cause of space as better than this Earth the author argues that man must be persuaded to rid himself of his "planetary chauvinism" and accept that even the Moon or Mars, for example, would not provide as suitable an environment as man-made habitats situated at one of the semi-stable Lagrangian points in the Earth-Moon system.

Three stages of development are suggested. "Island One" would be constructed primarily of aluminium and glass inside a sphere of 0.5 km diameter. The habitat, filled with an oxygen atmosphere, would rotate to provide an Earthlike gravity at the equator. The great majority of materials for the construction would be mined and transported from the lunar surface. Heavy industry, located nearby outside the sphere, and agriculture would, it is claimed, benefit from the continuous and predictable supply of solar energy, and the whole system could support a population of 10,000 in some comfort. Careful screening would prevent any unwanted "bugs" from being taken in from Earth; and presumably no malign mutation is expected to occur.

Within a further 50 years, the scale of development from these small beginnings is envisaged as leading to the "Island Three" habitats, cylinders 4 miles in diameter and 20 miles in length, maintaining several million inhabitants. There is probably no reason to dispute that such a design is already within the limits of present-day structural materials. Economic motivation for such colonies is claimed to lie with the construction of large solar power stations, subsequently placed into geosynchronous orbit and providing the Earth with electricity through high power microwave transmission. Such systems have, of course,

already been the subject of detailed design studies under NASA sponsorship, but it is not clear why sunlight in space should be more economic than in the desert areas of the near tropic latitudes here.

This book may properly rank as space fiction rather than prophecy but if so it is not because of any violation of scientific laws. No fundamentally new physics is required nor any great projection of present-day engineering practice. The speed with which such plans could be formulated and implemented even if adequate political backing was forthcoming, as suggested by O'Neill, is a point on which opinion will, however, vary greatly. In particular, there is little discussion of the inevitable sociological, legal and personal, including moral and religious, questions in their widest sense.

Much space is devoted to the projected economic aspects of space colonisation, particularly in terms of the initial investment, and to considering the viability of selling power to Earth through the solar power stations, considerations inevitably of a very speculative nature and, in themselves, hardly a complete justification for this mammoth scheme.

In chapter 2, entitled "The Human Prospect on Planet Earth", O'Neill presents what he sees as the most compelling arguments for a massive

colonisation of space. The shortage of land and energy on Earth are even now an obvious cause for concern and, with the expected rate of population growth, the situation may become desperate even before the middle of the next century. It may be thought, however, that these are basically political and ethical questions, that given good will, unselfishness, honesty, education, tolerance, sympathy and so on, it would be no more difficult to build a heaven on Earth than a "New Earth" (the title of chapter 4) in the heavens.

The New English Bible translates an aspiration of the early Christian church from two millenia ago, and no doubt echoing a far older yearning of many people, as "we . . . look forward to new heavens and a new Earth, the home of justice". This problem with its associated need for wisdom and moral fibre outweighs all the technical difficulties we face. Solve this and we might avoid the energy crisis of the late 1980s and the serious risk of war between "haves" and "have-nots" before the end of then century. □

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## Animal cells in culture

*Biochemical Methods in Cell Culture and Virology.* By Robert J. Kuchler. Pp. ix+331. (Halsted/Wiley: New York and Chichester, UK, 1977.) £22.50; \$38.

BECAUSE research in animal virology is based to a large extent on the use of animal cells in culture, it might seem a good idea to include in one manual the procedures of both. In fact, the task is not an easy one, as this review will suggest.

The stated aim of this book is to provide a guide for growing, handling and studying viruses and cell cultures in the hope that the student considering a career in virology, cell biology or cancer research should find it useful. There are three parts, of about equal lengths: Cell Culture, Virology, and

Macromolecular Analysis. The content is essentially a collection of very many procedures. Each procedure, or group of related procedures, is preceded by a brief introduction on its purpose and, very cursorily, on its history.

Not many students can secure a copy of the standard procedures, formulations, recipes, and so on, which several large research laboratories, dealing with virology and cell cultures, necessarily keep. These procedures are often collected in loose-leaf Laboratory Manuals. Every time that a procedure or formulation is changed, it is an easy matter to replace the corresponding pages.

The next best method (though expensive) for a student who cannot get hold of any such manual, is a book; but a book does not have replaceable



loose pages. In the two fields of virology and cell culture the speed of advance of methods is very fast, much faster than that of ideas. Thus, a manual on methods requires continuous updating.

Even the short time between going to press and publication has had this undesirable effect. Two examples: the procedures for cell fusion stop at the (doubtfully practical) use of lysolecithin (1972); that is, before the introduction of polyethyleneglycol; and in the section on "Macromolecular Analysis", sequencing of viral DNA genomes by means of restriction endonucleases is not even mentioned.

This book thus falls between two stools. As a manual on methods it is, inevitably, already outdated in parts and will very soon become extensively so. As a general survey of viruses as probes for cell biology its aim is not

wide or high enough. Perhaps fewer methods, just as examples, but a much deeper and wider discussion on the current problems of virology and on how those methods contributed to solving them, would have made the book more stimulating. The student is much more helped by making him interested enough to look up the literature on procedures than by providing him with ready-made recipes of very short half-life. As it is, this book will be more valuable as a source of references in the libraries of research laboratories than as a laboratory manual at the bench, or as reading for postgraduate students.

**G. Pontecorvo**

*G. Pontecorvo recently retired as a member of the research staff at the Imperial Cancer Research Fund Laboratories, London, UK, and is now Honorary Consultant Geneticist there.*

## Mammalian fertilisation mechanisms

*Fertilization Mechanisms in Man and Mammals.* By Ralph B. L. Gwatkin. Pp.x+161. (Plenum: New York and London, 1977.) \$21.

FERTILIZATION is intrinsically a tremendously important process, marking as it does the union of male and female gametes, the recombination of their genetic loads, and the initiation of the new individual. Only in comparatively recent times, however, have various aspects of fertilisation in mammals become susceptible to direct experimentation. The earlier work was consequently restricted largely to non-mammalian forms, notably the marine invertebrates. This area of investigation has continued to be most productive and is now experiencing a recrudescence of research interest, which, together with studies based on the new capabilities in the handling of mammalian gametes, is yielding a wealth of new information. The time is ripe for an up-to-date and critical collation of published data from both mammalian and non-mammalian sources. Gwatkin's book goes a long way to meeting this challenge and should certainly fill a need.

Chapters in the book deal with the development and properties of the gametes, their transport in the female tract, fertilisation *in vitro*, sperm capacitation, the acrosome reaction, sperm penetration of the zona pellucida, sperm-egg fusion, the defence against polyspermy, pronucleus formation and the metabolic events detected during fertilisation. Additionally, atten-

tion is given briefly to the fate of surplus spermatozoa in the female tract and their interaction with somatic cells, and to the results of recent work on parthogenesis. There is also an Epilogue in which the author indicates directions for future research.

In the relevant chapters, particular attention is given to the exploration of the surface properties of gametes using lectins and other agents, and the close study of the successive stages of interaction of egg and spermatozoon which betoken different orders of response. A good deal of mystery still attaches to these processes and this in itself should serve as a powerful stimulus to further research. A fascinating feature of this phase of fertilisation lies in the apparent duplication of gamete properties and faculties, which denotes a curious limitation in the forms of substrate on which the influence of evolution has had to operate. The consolation for the biologist lies in the possibility of drawing useful inferences from what can be regarded as complementary patterns.

The book is written in a clear concise style and bears the authoritative stamp of an active and highly productive investigator in the field. The wide range of the author's acquaintance with the literature is evident throughout the text and in the extensive list of references provided.

The book can be warmly recommended to all those involved in research on gamete physiology and fertilisation, in non-mammals as well as mammals, and as an essential source of information and ideas for advanced students of medical and biological sciences.

**C. R. Austin**

*C. R. Austin is Charles Darwin Professor of Animal Embryology at the University of Cambridge, UK.*

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## Logical foundations of computation

*The Theory of Computer Science: A Programming Approach.* By J. M. Brady. Pp. xiii+276. (Wiley: New York and London, 1977.) Hardback £8; paperback £4.50.

BRADY has written a book which can be warmly recommended to readers wishing to understand the logical foundations on which computation rests. It will be particularly valuable to students of computer science, but may be of interest also to computer programmers who wish to widen their horizons.

As a practical discipline, computing grew up in the late 1950s and early 1960s. Recruits to the programming profession came from mathematics, engineering, science, and even the classics and humanities. These people applied a largely intuitive approach to solving problems pressed upon them by commercial and other users, urgently requiring effective solutions. There were many difficulties, and many projects were late in completion, cost more

than was intended, or were even aborted before completion. The lessons learned during this period led to the formulation of a set of methodologies for program design and construction which are now often graced with the name "Software Engineering".

To aid the newly emerging discipline, theoretical foundations have been and are being developed. Methods of defining the syntax and semantics of programming languages, methods of proving the correctness and equivalence of programs, and of analysing the complexity of algorithms, are theoretical developments with immediate potential impact.

Arising, however, from the mathematics and logic camp there has come a theoretical background to computing which is often criticised as quite irrelevant to the practising programmer. Brady argues that this is due to a misunderstanding of its position in the logical structure of the subject. The theories of computability, based on Abstract (Turing) Machines and General Recursive Functions belong to the meta-theory the goals of which are to define precisely the meaning of 'computable' and to discover the theoretical limits of computability.

His book is accordingly divided into

two parts. Part 1 covers the meta-theory, whereas part 2 (which has a different title in the contents list and in the text) discusses the approach to a theory of computer science.

Brady does not assume extensive mathematical knowledge of his readers, and the prerequisites are summarised in an appendix. Throughout the book he leans heavily on the intuitions of the reader as an experienced programmer. He compares his approach with that of M. L. Minsky (*Computation: Finite and Infinite Machines*, Prentice-Hall 1967) by suggesting that his book is to programming what Minsky's is to hardware; hence the second part of his title. He writes with an enthusiastic style which makes his text very readable. There are frequent invitations to the reader to extend his understanding by proving lemmas and solving problems. These he calls "checkpoints", a usage which I find irritating. There are also more typographical errors than one expects in a book of this quality. These are, however, minor blemishes in an otherwise excellent book.

S. J. Goldsack

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## Neutrino proceedings

*Proceedings of the International Neutrino Conference, Aachen 1976.* Edited by H. Faissner, H. Reithler and P. Zerwas. Pp. xii+748. (Vieweg: Braunschweig, 1977.) DM168.

It has been interesting to review this conference report while attending the 1977 Lepton-Photon Symposium in Hamburg, which covers the same field and much more. Rather than making last year's news seem old and dull, this year's conference illustrates how useful the Aachen proceedings can be as a reference. Aachen was a specialised conference and the speakers had time to do their experiments and theories justice. At conferences with a wider spread of subject matter the results can be reported but the details are lost.

Some of the results reported at Aachen have, of course, now been overtaken by better experiments, or modified by more careful analysis. Nevertheless, much of the picture is the same as it was last year. The four-quark theory, with charm, still stands and a great deal of the evidence for this is reported in these proceedings; including a few wisely chosen non-neutrino results from  $e^+e^-$  storage rings. The quark-parton picture of

hadrons is being increasingly refined, with about the correct amount of scale-breaking to satisfy the asymptotically free gauge theories. As well as data on these effects there is a useful review by the late Benjamin Lee, who includes a simple introduction to scale-breaking in the course of his final summary of the whole conference.

Theoretical contributions from Dalitz, Cabibbo, Salam and others give a picture of the variety of different quark models still available. M. K. Gaillard and de Rujula discuss the success of the charm concept in the light of the rich spectrum of particles reported in the experimental sessions. In contrast to their confidence, there are calls from Wolfenstein, from Fritsch and from Sakurai for more data on neutral currents, on second class weak currents, on neutrino oscillations, on parity violation in atomic physics, and so on. We certainly do not yet understand the weak interaction completely.

As well as high energy accelerator physics, there are a few papers on neutrino astrophysics and on assorted low-energy topics such as double  $\beta$  decay, and the  $^3\text{H}$  spectrum as a probe of the neutrino mass.

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## Prosimian behavioural ecology

*Ecology and Behaviour of Nocturnal Primates.* By P. Charles-Dominique. Pp. x+277 (Duckworth; London; Columbia University Press: New York, 1977.) £12.50; \$21.90.

THE behavioural ecology of the prosimians, an essential element in our understanding of the evolution of the order of Primates as a whole, raises special problems not encountered in field studies of monkeys and apes. The majority of prosimians are small, shy inhabitants of dense forest environments, solitary in their active phase and, most pertinent here, nocturnal. The task of adequately studying these in the wild is daunting and might seem impossible at times. Yet this book is a measure of just how successfully that task can be carried out.

In the main, it presents the results of almost a decade of research in Gabon in West Africa on five sympatric species of lorid (three bushbabies, the potto and the angwantibo) living in the equatorial rainforest. Covering in great detail such areas as feeding and diet, stratification, locomotion, defence against predators, and many aspects of social behaviour and demography, it is concerned not simply with each species viewed in isolation but with the vital questions arising from their sympatry. It succeeds in shedding considerable light on the evolution of this group, the adaptive strategies called forth by the particular and precise ecological niches they occupy, the specialisations of structure and behaviour that have developed, and especially the manner in which competition between the species has been minimised.

In most instances, penetrating and wide-ranging analysis leads to well-rounded and convincing synthesis. This has been achieved in two ways: first, by the skilful use of a variety of field techniques, ranging from direct observation with headlamp to capture-mark-release procedures and radio-tracking, the latter yielding some most valuable information. Second, the author repeatedly recognises or finds confirmation of his emergent hypotheses in acute 'casual' observations of untrammelled animals, or in beautifully simple manipulations of the forest environment or captive circumstances.

All this is not to say that Charles-Dominique's account of the Gabonese prosimians is complete or without fault. In places, a paucity of hard data, unrevealed sample sizes or the absence of detail when procedures are des-

cribed, raise doubts in the mind. The repertoires of communicatory and some other behaviours are incomplete and the social significance of play in lorids is totally overlooked. Moreover, certain interpretations are suspect or erroneous—for example, that concerning the defensive posture of *Arctocebus*, and the view that the primary function of the prosimian dental comb is gum collection rather than grooming, despite the indisputable importance of the former function in *Euoticus*, so clearly shown here.

The title, if not the subtitle of the book could be misleading if, by it, one were led to expect a thorough-going review of our knowledge of all nocturnal primates. In this respect, the work is erratic and there are some surprising omissions, even within the narrower compass of the Loridae. A concluding chapter does attempt to place nocturnal primates among the diurnal forms in a wider perspective, but here again the

quality of the result is decidedly uneven, marred by the dubious validity of a number of contentions regarding Primate evolution and ecology. Thus, the idea that competition for food is necessarily eliminated or even reduced by a Box-and-Cox temporal partitioning of an ecological niche does not stand up to close scrutiny as a general truth.

The strength of this book, and it is indeed strength, lies in the author's approach to, and resolution of, the problems of behavioural ecology in his Gabonese prosimians; and in his demonstration that no aspect or detail of structure or behaviour is arbitrary, without meaning in terms of the animal's survival. That is reason enough to commend this volume.

Gilbert H. Manley

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## Sound waves in solids

*Sound Waves in Solids.* By H. F. Pollard. Pp. 366. (Pion: London; Academic: London, 1977.) £10.50.

THE author of this latest addition to the rapidly growing literature of acoustics is Dr Howard F. Pollard who is an Associate Professor in charge of the Physical Acoustics Laboratory at the University of New South Wales in Sydney, and is the leading Australian worker in this field. The objective of the author, as indicated in the introduction, is to provide initially the necessary theoretical background for the reader to appreciate fully the various applied fields of acoustics, such as non-destructive testing. This practical aspect is kept well to the fore throughout the book, and uniquely the author introduces, after the basic theory, synopses of experiments which appropriately illustrate the problem under discussion. A particular usefulness of the text to the student is the provision of sets of questions at the end of each chapter which are based on the problems included at the end of specified sections of the foregoing subject matter. A full set of solutions to the book.

In considering the general contents of the book, it is the chapter on acoustic waveguides on which the author can be particularly congratulated. He has introduced this important topic through the simpler system of a fluid waveguide, and, having established the essential theory and con-

cepts, he deals with the understanding of the various practical applications. The chapter on ultrasonic experimental techniques is quite comprehensive and the latest developments such as ultrasonic spectrometry receive adequate recognition. The author's own research involvement in such work enriches his presentation of the subject; this also applies to the chapter in the book concerned with the exciting field of acoustic visualisation. A representative number of holographic applications are given, and here the author has revealed his musical interests by the inclusion of some reconstructions at audiofrequencies.

The problem of the choice of references is difficult in a field like solid-state ultrasonics, which is large and rapidly expanding, but the author has helped the reader by including the appropriate references at the end of each chapter. Moreover, those concerned with particular experiments are given with the text. An author and a subject index are also included. The line diagrams are very clear and also the text, although the use of smaller print for experimental descriptions gives an impression of over compression.

The title is indicative of the breadth of the subject so efficiently and clearly discussed by the author, and the book deserves to be welcomed alike by students, teachers and research workers.

R. W. B. Stephens

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# newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. The Reader Enquiry Card faces the inside cover.

**Microprocessor-controlled logger.** Pye Unicam. The Philips PM 4000 system is designed for logging voltages, currents and temperatures; later models will handle mechanical (strain) measurements directly. Up to 950 channels can be controlled by one unit, and remote control and operation of the data logger are possible. The main-frame unit can hold up to 50 channels with extension units for each additional 100 channels. Extension units can be up to 10 m from the control unit—the use of digital communication between mainframe and extension units eliminates noise problems. Results can be conditioned into the relevant engineering units using internal linearisation tables and formatted for internal or external presentation. Programming is reduced to a simple question-and-answer dialogue using a built-in keyboard and integral alphanumeric display, and no special programming language is necessary. Completed programs can be dumped out to, or dumped in from, a digital cassette recorder enabling custom program libraries to be built up.

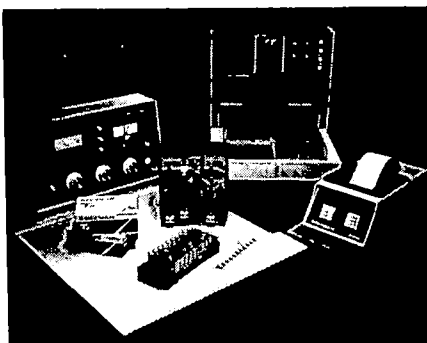
Circle No. 45 on Reader Enquiry Card

**Single-test creatinine analyser.** Beckman. The new Creatinine Analyzer-2 automates precise measurements of creatinine in plasma, serum, urine, or amniotic fluid. Time from sample injection to final readout is 26 s compared with the 15 min usually required for running the test manually. Creatinine is clinically important in determining kidney function, in monitoring renal dialysis therapy, and as a warning of incipient muscular disease such as muscular dystrophy and hypothyroidism. Operation of the Creatinine Analyzer-2 is based on the Jaffe reaction. Colour is generated as the patient sample mixes with reagent under controlled temperature conditions, and the analyser measures the rate of colour change, which is a more precise indicator of creatinine than endpoint analysis.

Circle No. 46 on Reader Enquiry Card

**Sample-handling system for radioimmunoassay.** Beckman. The modular Phase I RIA sample-handling system is designed to improve radioimmunoassays by simplifying procedures for rapid, accurate, inexpensive analyses. Stand-alone instruments, accessories, and packaged RIA kits make up the system, which is available as a whole or in parts. Phase I system includes the Gamma 4000 gamma spectrometer, which is capable of processing up to 400 samples automatically; an on-line DP-5000 microprocessor data reduction system, which stores up to 10 RIA data reduction programs including counts per minute; the Model J-6 Centrifuge; and an all-in-one pipettor/diluter/dispenser which allows the solid-phase Beckman RIA Kits to be run with only one simple pipetting step. Basic to the Phase I System are Beckman double-antibody, solid-phase RIA kits, which reduce incubation time, centrifugation requirements, and chance for error.

Circle No. 47 on Reader Enquiry Card



Beckman RIA system

**Blood bank refrigerator.** Forma. The Forma Scientific model 3880  $\pm 4^\circ\text{C}$  blood bank refrigerator has a 300-bag capacity and features top-to-bottom temperature uniformity within  $0.5^\circ\text{C}$  in compliance with nationally recognised standards. Standard equipment includes six 50-bag capacity roll-out storage drawers with label holders, a self-charging battery alarm system with alarm ringback and remote alarm contacts, a pressure writing temperature recorder, automatic condensate removal (to eliminate drain lines), and a 100% stainless steel interior cabinet with cabinet lights. The unit is 30 inches wide, 28 inches deep, and 73 inches high.

Circle No. 48 on Reader Enquiry Card

**Thermomechanical analyser.** Perkin-Elmer. The Model TMS-2 thermomechanical analyser when used for the thermal characterisation and quality control of materials, determines precisely and rapidly, dimensional changes (expansion and extension modes) or visco-elastic changes (penetration and compression modes) of small samples in any form—powder, pellet, film, fibre or moulded part. The Model TMS-2 system consists of analyser, analyser control and heater control modules. Displacement can be recorded simultaneously as either integral or derivative signal, both individually attenuated and filtered. The linear variable differential transformer is in complete thermal isolation from the furnace, which permits operation at very high sensitivity with flat baselines over a broad temperature range. Heating and cooling rates from  $-170^\circ\text{C}$  to  $+325^\circ\text{C}$  are possible with the standard furnace and an optional low temperature furnace is available. A sliding furnace assembly, positive positioning of probe on sample and open tube design facilitates ease of sample handling.

Circle No. 49 on Reader Enquiry Card

**Automated blood gas analyser.** Technicon. The Technicon BG II, pH/blood gas system uses an advanced, built-in computer to help provide accurate answers on eleven parameters on blood samples as small as  $130\ \mu\text{l}$ . Small samples give the instrument wide application in hospital critical care facilities where patients with severe burns and other major illnesses are treated. These patients, as well as infants and the very elderly, have frequently been incapable of giving a large enough sample in the past. The Technicon BG II system is extremely easy to operate and maintain. With the BG II, the hospital can have cost-effective coverage in the central laboratory as well as in any other areas where blood gas samples are processed. The results of the eleven tests are available in  $2\frac{1}{2}$  min, and are printed out on paper tape or multiple-copy ticket. The values for pH,  $\text{PCO}_2$ ,  $\text{PO}_2$ , and haemoglobin are measured directly: standard base excess, base excess, standard bicarbonate, bicarbonate,  $\text{O}_2$  saturation,  $\text{O}_2$  content, and total  $\text{CO}_2$  are computed and printed out simultaneously.

Circle No. 50 on Reader Enquiry Card

**Electronic precision balances.** Mettler. A compact, rugged housing is used for two new electronic precision balances. The PL1200 can weigh up to 1,200 g with a readability of 0.01 g and the PL200 can weigh up to 220 g with 0.001 g readability. An automatic stability detector circuit monitors the zero point and indicates when a reliable weighing result is available for reading. The measuring cycle can be adjusted from the outside in four different steps. This allows the balance to adapt to difficult weighing problems (such as restless lab animals) and to prevailing environmental conditions without affecting its weighing performance. The capabilities of weighing below the balance and of measuring moisture loss with the help of the LP12 infrared dryer are also built into these balances. Printers, weight-count converters and other peripheral instruments can also be connected by means of a BCD output.

Circle No. 51 on Reader Enquiry Card



Mettler PL1200

**Interferometer.** Metals Research. The new Metals Research interferometer has been developed for the examination of the transmission characteristics of laser rod materials both for zonal selection and final quality assessment. Following the classical lines of the Twyman-Green Interferometer the instrument is packaged so as to provide a sample space of 40 mm diameter, 200 mm long. Folding the light path in an optimum manner gives a table-top instrument of only 20×11×10 inches, weighing approximately 110 pounds. Accuracy is 1/20 wave. Special design features include built-in laser illumination to provide high contrast fringes for critical viewing of small apertures (6 mm dia.), and a decohering filter (rotating disk) to suppress spurious fringes created within the system. A built-in Polaroid roll film holder takes 1:1 photographs of specimen patterns.

Circle No. 52 on Reader Enquiry Card

**Automatic analyser for nitrogen oxides.** Pye Unicam. The Philips PW 9762 analyser continuously and automatically measures atmospheric oxides of nitrogen (NO, NO<sub>2</sub>, or their sum NO<sub>x</sub>) in a simple and economic manner, and it will do so for up to 3 months without intervention. NO and NO<sub>x</sub> are both measured in identical samples of ambient air, NO<sub>2</sub> initially being qualitatively reduced to NO for the measurement of NO<sub>x</sub> (NO<sub>2</sub> is the difference between measured values of NO<sub>x</sub> and NO). This use of identical sampling eliminates false negative values of the NO<sub>x</sub> signal. The PW 9762 makes use of the chemiluminescence principle which creates a reaction between NO and O<sub>3</sub> and, by providing an in-built ozone generator, the Philips analyser obviates the need for an external supply of gas—such as, for example, oxygen. Highly-sensitive pulse-counting techniques enable the analyser to be used in normal atmospheric-pressure conditions, thereby overcoming one of the earlier drawbacks of the chemiluminescence principle which demanded near-vacuum working conditions. The PW 9762 is extremely compact and fits into a standard 19-inch racking system. It incorporates its own sample pump and power-supply unit and can be ready for use within 20 min of connecting up.

Circle No. 53 on Reader Enquiry Card

**Anaerobic work centre.** Forma. Forma Scientific introduce a completely self-contained anaerobic work centre designed to add immediate anaerobic capabilities to clinical and research labs of all sizes. The work centre consists of a large-capacity glove cabinet with a highly visible work area; a built-in incubator with solid-state temperature control and a forced-draft air flow system to maintain a comfortable working environment; and a front access auto-sequence interchange with single, push-button aerobic to anaerobic conditioning for quick-in, quick-out transfer of anaerobic cultures and apparatus. The work centre is complete with regenerative palladium catalyst and dessicant dryer modules, de-ox indicators, disposable work pads, and a waste removal system. Accessories available include an anaerobic collection/transfer cart, add-on work modules, inventory racks, and a mobile instrumentation bench for set-up of an integrated anaerobic station, including a low noise, oilless vacuum pump, built-in CO<sub>2</sub> and N<sub>2</sub> gas bottle platform, undercounter storage cabinets, and dual-wheel casters for easy moving.

Circle No. 54 on Reader Enquiry Card

**On-line chemical analyser.** Kevex. The Kevex material analyser is intended for rapid and non-destructive on-line measurement of chemical compounds. The analyser includes a pre-programmed microprocessor capable of calculating the concentrations of up to 19 different elements simultaneously in as little as 5–10 s. Applications include routine quality control of fluids and slurries by batch sampling tests or continuous on-line flow analyses with the option of closed-loop control. Provisions for data logging and external signals for initiating element concentration correction or product rejection are included. The system can be fully automated.

Circle No. 55 on Reader Enquiry Card



Kevex material analyser

**Low-temperature temperature controllers.** Neslab. Exatrol and Cryotrol controllers are designed primarily to extend the usefulness of the Neslab immersion coolers by providing the ability to maintain constant temperatures at any point up from the lowest point attainable. Neslab CryoCool and Bath Cooler immersion coolers can now be operated to maintain constant temperatures from as low as -100 °C. The Exatrol temperature controller offers temperature stability of better than ±0.05 °C when used with a Neslab CryoCool or Bath Cooler. Linear cooling or heating rates can be programmed by adding the ETP-3 electronic temperature programmer. The Cryotrol model provides temperature stability of ±0.5 °C. This economical method can be used where less critical temperature control is needed. A constant low temperature bath, as well as programmed freezing rates, is now possible with this new series of temperature controllers.

Circle No. 56 on Reader Enquiry Card

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## Mr Desai takes on the scientists

USER departments, more relevant research, national needs . . . familiar words in Britain during the period of the Rothschild reorganisation, familiar words more recently in Australia during attempts to dismember the the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Now the Indian government of Mr Morarji Desai is going through a similar operation with its own large scientific body, the Council of Scientific and Industrial Research (CSIR). And the opposition to these moves from the Indian scientific community is extremely vocal.

CSIR is an organisation very largely conducting applied research. First established in 1942, it is most closely associated with Mr Nehru who put it on its present firm footing in 1952 and ensured, by making the prime minister its president, that the organisation had a substantial degree of autonomy, in that its director general could by-pass ministerial bureaucracy. Furthermore, Nehru made himself minister for science, and in recent years Mrs Gandhi has also held that post, so there have been the clearest signs to Indian scientists of the importance that the government attached to science and technology. In these past thirty-five years CSIR has blossomed into an organisation with forty-four laboratories including ten cooperative research associations.

The days are gone, however, when applied scientists could claim total freedom from direction. And the arrival on the scene of governments like those of Edward Heath, Malcolm Fraser and now Morarji Desai to find large expensive autonomous organisations nominally completely decoupled from departments working in the same field was bound to trigger off some very serious questioning. In cold logic many of CSIR's laboratories could as sensibly be constituent of ministries such as industry, energy, electronics and agriculture. Thus the dismemberment of CSIR with up to 28 laboratories detached to user-ministries is not without a rational basis.

Against this, however, some very serious arguments must be ranged. First, there seems to have been no consultation of scientists within CSIR before the cabinet made its decision—and thus no opportunity for the

sort of debate which might well have thrown up alternative structures. Second, CSIR laboratories are at present expected to be accessible to government and industry alike, whereas once in the control of a ministry access might become much more restricted for those not within a tight circle. This is an interesting point which in the British context would certainly be worth studying. Third, ministers with tame scientists may be tempted to use these scientists simply to bolster up their own prejudices and not for objective assessment. Fourth, India leads the world in ministerial bureaucracy and red-tape (thanks to the British), so any move that puts scientists under the control of so many paper-shufflers and rule-book-followers has to be regarded with alarm.

These are weighty objections which in the British situation were only alleviated by the placing of chief scientists of intellectual distinction right in the midst of the relevant ministries. In India, the path to new machinery may well be different but for certain it must take note of the concerns of scientists. This has not yet happened, and the government will find itself with a very dispirited bunch of scientists if it does not bring them more into the discussion.

A rather different objection comes from those who fear that the dismantling of CSIR is one stage in the process of reducing India's aspirations to technological self-reliance. CSIR has pursued many scientific projects specially aimed at cutting down the need for expensive imports, and anything that rewards the organisation for this by splitting it up is bound to be viewed with great suspicion. The Indian National Science Academy says this is an elliptical way in a statement which recognises the benefits of some foreign technology but points to the immense pools of talent available. The press have taken it up more stridently in statements such as 'CSIR has been an eyesore to the multi-nationals', and 'CSIR is the first martyr to the Janata Government's ill-concealed solicitude for the international giants'.

All this adds up to a compelling case that the government should come out and debate its proposals openly. Once CSIR is in pieces a major national asset is never likely to be put together again. □



# Science versus safety: who should judge the balance?

*R. N. P. Sutton, of the Department of Virology, Wittington Hospital, Manchester, offers some reflections on the possible implications of increasingly stringent health and safety requirements for scientific research in Britain*

EMMA LIVRY was a young and talented dancer in the Paris of the 1860s. She chose to ignore the management in a question of non-flammable material and, in consequence, was fatally burned during a performance at the Opéra. Mlle Livry weighed art against safety and paid the price.

I doubt whether such a conscious choice could be made today. Over the years, safety regulations have proliferated, culminating, as far as Britain is concerned, in the Health and Safety at Work Act of 1974. These regulations have mandatory force and, whether *post hoc* or *propter hoc*, we now have a universal obsession with safety. Living and working in a scientific community, do we expect too much when we hope that a fair balance can be struck between a crippling devotion to safety-first on the one side and a disastrous *laissez-faire* on the other? For the moment, let us confine ourselves to microbiology, for that is the field with which I am familiar.

Do laboratory workers handle pathogenic organisms carelessly? I hardly think so. In the diagnostic field, all are well aware of the dangers of hepatitis, tuberculosis and so on, probably more so than our colleagues in other disciplines. There has been much thought about the newer hazards, Lassa fever, Marburg and rabies viruses, and the risks in handling these agents are now well recognised. Special control measures to prevent the spread of infection by these and similar agents have been set up in Britain. The Dangerous Pathogens Advisory Group (DPAG), a body broadly analogous to the Genetic Manipulation Advisory Group (GMAG), has laid down procedures for the handling of these Category A pathogens. The procedures apply to laboratories which, as a matter of deliberate policy, hold or handle or might in future hold or handle these pathogens.

In laboratories with diagnostic responsibilities there are unavoidable risks in the handling of specimens. Where large areas, maybe including major seaports or international airports, are served, the chances increase of fortuitous encounter with dangerous pathogens. Facilities are required for safe handling of specimens from patients—and, indeed, the safe handling of the patients themselves—in whom exotic infections such as Lassa fever are suspected. At this stage, the practicalities of administration and finance become paramount. The necessity of expensive preparation for a rare event does not appeal to cost-effective administrators, hard-pressed by other and urgent appeals. There is a temptation to purchase a facade of equipment and, perhaps, to designate an empty room as a high security area. Justice, but not full justice, is seen to be done. In circumstances such as these, limited by unavoidable financial constraints, clinicians, nursing staff and laboratory workers may be edged into positions which are unacceptably dangerous.

But is not all this within the domain of the Health and Safety at Work Act? Here, it is expressly laid down as a duty that the exposure of persons to risks to their health and safety is forbidden. Yes, but . . . presumably, the legislators did not intend this Act to be read as forbidding nurses to dress infected wounds, laboratory workers to test

infected specimens or doctors to attend to patients with contagious diseases.

Yet dangers exist and there would seem to be practical and financial difficulties in implementing the provision of safe conditions of work, in particular with respect to the rare, but real, hazards presented by Lassa fever and the like to the ordinary hospital and laboratory of any size. Implementation of the spirit of this law is much easier when we consider the planned investigation of known infectious agents. Here, we are often on different ground, and a fable comes to mind.

In a far away land, there was a tragic disease, well known to medical men. Rumours claimed that this disease could be transmitted to monkeys and two doctors decided to try the experiment. One told his colleagues about the exciting prospects of his investigation; these friends banded themselves into a committee and prudently forbade him to handle such a dangerous pathogen. The second doctor kept his counsel and success came his way.

I am not sure whether this fable could be held to apply to any known disease. Told twenty-five or thirty years ago, it might have applied to poliomyelitis: told today, to Creutzfeldt-Jakob disease. Times change, and it is interesting to speculate on what would have happened if the early work on poliovirus had been carried out in today's climate. Would we now have a poliovirus vaccine or would the experiments have been terminated, for the safety of the laboratory workers? Either way, patients with poliomyelitis would still have been cared for and pathological specimens subjected to routine examination without much regard for the letter of any safety legislature.

In the past, experimental risks were assessed by the scientist concerned. Accepted as being in possession of the facts, and capable of marshalling them with responsibility, his decisions were given due weight. Today this is not so and we have a society where everyone questions authority and claims the right to speak and act, often on topics beyond their competence.

In this way, bodies often effectively lay in composition can inhibit individual workers through decisions which are arrived at by processes which are essentially non-scientific (risk avoidance, responsibility spreading and undue reliance upon public opinion). Such decisions, although they may well implement legislation such as the Health and Safety Act, may equally well not be in the public interest (that is "Doing today those things that men of intelligence and goodwill would wish, five or ten years hence, had been done"—Edmund Burke).

How can we strike a fair balance between the demands of safety and those of the public interest? My concern is by no means theoretical and I know personally of recent instances where local bodies, acting in their own wisdom, have attempted, in the name of safety, to ban certain microbiological experiments. On both occasions, as it happened, the offending agents were slow viruses or possible slow viruses. Next year, perhaps, influenza virus vaccines will take over the role of bogeys for the timorous in the local safety committees—who knows?

A solution may lie in changing slightly the role of the DPAG. In the few programmes involving genetic engineering, control is rightly in the hands of the GMAG. In the many experiments involving micro-organisms, the ultimate sanction in questions of safety and the power of veto should be, not in the hands of local bodies, but in those of the DPAG. In this way both the public safety and the freedom of the investigator would be protected. □



# Health and safety 3 years on

Alastair Hay traces the teething trouble of the UK Health and Safety Commission since its inception in 1974

WHEN the UK Health and Safety at Work Act (HSW Act) received the Royal Assent on 31 July 1974, a single agency—the Health and Safety Commission (HSC)—was brought into being. It was charged with securing the health, safety and welfare of people at work, and of the public out of work. The Commission has a Health and Safety Executive (HSE) to implement its decisions and coordinate the safety work previously carried out by separate agencies dealing with occupational safety and health, explosives, nuclear installations, factories and employment medical advisory services. Three other agencies were also allocated to the HSC and, in spite of considerable pressure to return them to their former status, have been retained by the Commission. They are the inspectorates for mines and quarries, farm safety, alkali and clean air.

The HSW Act states that the Commission shall consist of a chairman and "not less than six nor more than nine other members" to be appointed by the Secretary of State for Employment. At the moment the Commission has eight other members. Employers' organisations in the form of the Confederation of British Industry (CBI) appointed three, the Trades Union Congress (TUC) nominated a further three, local authority organisations two more. Although several nominations have been considered for the ninth post no single nominee has proved acceptable to both employer and employee representatives on the Commission. CBI nominees have been vetoed by the TUC representatives and vice versa. According to one member of the HSC this political infighting was only to be expected in the creation of a new organisation. It had in no way affected the work of the Commission which, he said, was "going well".

Evidence that both sides of industry are cooperating well on safety issues is provided by the safety representatives' legislation due to come into force on 1 October 1978. This provides for trades unions to establish 'safety watchdogs' in work places throughout the UK; 150,000 'watchdogs' are likely to be appointed, according to HSC estimates. When the measure was first proposed, CBI representatives opposed it, fearing that it would increase the power of trade unions; but, with no machinery other than the unions for negotiating with workers in industry, their opposition was short lived.

A further threat to the legislation was posed by expenditure cuts implemented by the government. In fact the cuts would have stopped the legislation had the CBI and TUC not put pressure on the government to secure the measure. By this time both organisations were agreed that the legislation was important. As the coal mining industry has had safety representatives at the work place for many years, the pressure is not entirely new. But its success in the coal industry is undoubtedly one reason for the HSC introducing it into others. It is also, say the HSC, the only way of ensuring that people can participate actively in decisions affecting their own safety.

## Placing responsibility

The 1974 Act is quite explicit when it places responsibility for safety at work; it is the duty of every employer to ensure, "as far as is practicable", that his employees are not "exposed to risks to their health and safety". The HSE's factory inspectors have the task of ensuring that the law is enforced in the factories of manufacturing industry in the UK. In the past these inspectors had to be generalists, each one being responsible for about 500 different premises. Today there is a different strategy: inspectors concentrate on specific problem areas. A new class of specialist inspectors is being trained who will be better able to advise local safety representatives.

These inspectors are well armed too. If any employer does not comply with an 'improvement order' an inspector can issue a 'prohibition order' to stop a particular activity. In the HSC's report for 1974-76, inspectors are reported to have served 5,433 improvement notices, 1,951 immediate, and 799 deferred prohibition notices. Of the 44 appeals lodged against these notices, 31 were withdrawn, some were modified but in no case was a notice cancelled. In the HSC's view this outcome is "very satisfactory"; the Commission's inspectors are using their legal powers, but with prudence.

Prohibition notices are unlikely, however, to be served on the directors of research laboratories in the UK. According to Audrey Pittom, the Director of the Hazardous Substances Division of the HSE, "enforcement or spot checks are not necessary for research institutions". In Miss Pittom's view, scientists obviously need to exercise care in the laboratory. But she ac-



VCM worker with personal monitor

knowledges that most scientists are usually well versed in the hazards involved in their work. She says that the HSE feel that it is the large scale production processes in industry that present the real dangers. It is in these circumstances, where there is a long chain of command with no single individual responsible for the whole operation, that problems arise; this, she adds, is not the situation in the laboratory.

In laboratories where the staff are organised in trade unions, safety representatives with legal powers will be appointed by October 1978. Many well run laboratories already have safety officers so this measure is unlikely to bring about any serious disruption. It will probably be those laboratories engaged in work of a multidisciplinary nature where scientists, adequately trained in one field, can venture into another, which will come under closer scrutiny. This is an area of concern for many laboratory safety officers who argue that the technicians employed in these laboratories are often unaware of the dangers they run, either because they have not been told or simply because their employer's don't know the hazards themselves.

## Genetic engineering

One of the HSC's proposals has been viewed as a serious provocation by sections of the scientific community. It concerns the Commission's all embracing definition of what should, or should not, pass for genetic engineering. The Commission feels that there must be regulations to cover this useful, but potentially dangerous research field. Its guidelines, put forward in a discussion document last year, were deliberately all-encompassing: in the words of a spokesman for the HSC it "did not want to leave anything out". The definition certainly did not do that. In fact one commentator remarked



ironically that the definition was so broad that it would even preclude consumption of a bowl of yoghurt.

But this was not the only response from scientists to these proposals. Reaction was often far more extreme. Some biologists attacked the recommendations with a fervour bordering on hysteria, principally, says the Commission, because they were not fully informed of the reasoning behind the proposals. The Commission feels that some over-reacted, others were initially slightly misguided, but that the majority discussed the recommendations rationally. Note has been taken of these discussions and the Commission is now proposing a more selective definition of genetic engineering. It has yet to be approved by the Secretary of State for Employment.

On the subject of industrial carcinogens, the HSC view is unequivocal; industry must take more effective measures to reduce the risks. Inspectors in the HSE point out that workers in specific industries are still developing cancer. More and better surveillance is necessary. The HSC uses as its guidelines the recommendations of the 59th sessions of the International Labour Conference on the prevention and control of occupational hazards from carcinogens.

As a first step to limiting the spread of carcinogens, the HSC is preparing a notification scheme for all new substances. Where the quantity manufactured in, or imported into the UK exceeds 1 tonne per year, advance information on the substance's toxicological properties must be sent to the HSC. Substances already in use before the introduction of the scheme are exempt from its provisions unless there is evidence that they present a particular hazard.

Included in the toxicological details will be information about potential carcinogenic properties. As industry will be required to do its own testing, this will have to be done according to a protocol agreed with the Commission. Animal studies are still regarded by the HSC as the most effective for determining carcinogenic potential. But as they are expensive, the Commission argues that they are not feasible as screening tests for large numbers of new substances; the short term tests now available are more practical. Two short term tests favoured by the Commission are the Ames test—for assessing mutagenic properties in bacteria—and *in vitro* cell transformation in cultured cells. It is the Commission's view that the results of these tests, as well as other factors such as chemical structure, nature of exposure and the number of workers exposed, should be considered in assessing whether addi-

tional tests are necessary. Some scientists point out that co-carcinogens are not covered by short term tests, and they add that some attention must be devoted to this problem.

The Commission is unlikely to ban substances, however, simply because they are carcinogenic. The activity of the carcinogen, its use in industry and its manufacturing process will be considered before resorting to a banning order. The Commission feels that cleaner manufacturing techniques could reduce the risk to workers from some carcinogens. Audrey Pittom cites the case of vinyl chloride monomer (VCM) as a substance, which can be "control(led) without prohibiting". VCM, an important polymer in industry but linked with the deaths from angiosarcomas of a former ICI employee and a worker at British Petroleum, is now subject to far stricter manufacturing procedures than existed in the past. Thus vinyl chloride, once even mooted as a potential anaesthetic, has escaped the prohibition notices served on some chemicals in the UK, such as  $\beta$ -naphthylamine and benzidine.

In the final analysis it is epidemiological evidence which is necessary for assessing the carcinogenic risk a substance carries for humans. To assist with this, the HSE, and TUC in particular, would like to see industry keep better general medical records of employees. A few specific industries do keep this information but the TUC feels that all industrial concerns should do likewise. Some argue, therefore, that it is almost certain that pressure will be exerted on the government in a year or two to introduce amending legislation to the HSW Act to secure this end.

#### Unlikely measure

A measure not likely to be adopted in the UK in the fight against carcinogens is the proposition put forward by doctors representing two German chemi-

cal giants—Hoechst of Frankfurt and Bayer of Leverkusen. The doctors suggested that older workers be employed in plants manufacturing dangerous chemicals on the basis that a more experienced workforce exercised more care in handling toxic substances, and that if there was a latency period of 20–30 years between first exposure and the development of cancer, these workers may be less at risk of developing cancer than their younger colleagues.

The companies made it plain that they had never adopted this practice themselves and a spokesman for Hoechst said that it might be possible to implement the decision in smaller factories, but not in one as large as his. Reaction to the proposal in Germany from the Ministry of Labour, members of parliament and trade unionists was understandably hostile; it is hardly likely to be adopted. In Britain, Mr Bill Macmillan of the Chemical Industries Association thought that the proposal was an "interesting approach" but he insisted that it was "not one we apply in Britain, nor is it one which as far as I am aware had ever been considered in this country". Spokesmen for both the HSE and one of the larger chemical unions in Britain said that the suggestion was "politically unacceptable". The real flaw in the Hoechst suggestion as far as the HSE spokesman was concerned, was that if there was a risk of someone being exposed to a carcinogen it would be impossible to restrict the substance merely to the factory confines and prevent other people in the environment being exposed.

Thus the HSC and its Executive have to look at safety measures from both a political and technical standpoint. The Commission has had to exert some political muscle to resist pressure from mining and agricultural interests to have their safety inspectors detached



Heat exchangers at VCM plant



from the HSC and reinstated in their former guises. Attempts to have the Alkali and Clean Air Inspectorate returned to the Department of the Environment have also met with little success. But this should come as no surprise, for few governments would wish to see their own creations dismembered so early in life, and the HSC

is after all a protégé of a Labour government, albeit its second term in office.

It remains to be seen whether future British governments take the demands for dismemberment more seriously. By that time, however, most of the arguments in favour of such a measure will no longer apply, for the various in-

spectorates will be more coordinated, and existing and proposed legislation will be more in tune. As for the 1974 HSW Act, the results of that legislation can only be judged some years from now. Many are in no doubt that the Act will be shown to have achieved its objective, that is, a safer working environment in the UK. □

## Protecting production or workers?

In November 1974, the British Society for Social Responsibility in Science reported in *Nature* on its work on vinyl chloride monomer. The Work Hazards Group of BSSRS sent *Nature* this update of its activities:

OVER the past three years, BSSRS has expanded its hazards programme with the aim of providing information to those directly at risk on the factory floor and to community groups directly affected by industrial hazards. We now publish *Hazards Bulletin* five times yearly which includes material on particular hazards, developments in health and safety legislation, legal cases and trade union struggles for health and safety in the workplace. Pamphlets on noise, oil, vibration and asbestos dust provide more comprehensive analyses of the effects of these hazards and how to fight them. Our hazards enquiry service now receives 50-75 enquiries per month. Local hazards groups work within trades councils, local trade union branches and community groups on health and safety issues, and we talk directly to safety representatives and shop stewards on day release safety training courses organised by concerned members of the trade union movement. This direct contact has been invaluable to our work.

While we have been developing our work at the rank and file level, worldwide concern about environmental hazards has increased. The enormity and horror of the disasters at Flixborough and Seveso, the struggle of the inhabitant of Minimata Bay to obtain compensation for the damage done by mercury poisoning, the poisoning of the state of Michigan by PBB, the militant demonstrations against fission reactors in France, West Germany and the United States, have all forced the scientific communities in industry and the universities to begin a more systematic evaluation of the hazards of old and new technologies. So far, unfortunately, this response has been grossly inadequate, reflecting a remoteness from the problem and a lack of fundamental concern for those who are directly at risk.

A conference on risk held at Imperial College, London, last May and organised by the Council for Science and Society (CSS) and the ensuing leaders and articles in *Nature* (19 May, 26 May) expressed fundamental differences of approach between our work and current academic and industrial considerations. The dominant note struck at the CSS conference and echoed in the pages of *Nature* is the need to guarantee production. Health and safety issues are secondary to the needs of maintaining and increasing production. While this approach is understandable from the point of view of those responsible for planning the economy it is unacceptable

to those directly at risk in the factories and the neighbouring communities. Significantly, trade unionists and community groups are virtually excluded from conferences such as the one organised by CSS where the issues are discussed and where policy begins to be formulated.

Aside from the inexcusable absence of those directly affected by the discussions and the decision making, there are fundamental problems when the emphasis is on guaranteeing production first and safety second. Such protection as is provided involves enclosing the worker in cheap protective clothing rather than enclosing or redesigning the production process itself. Industry sees the problem as protecting the operation and design of the process, an attitude which characterises a number of industrial approaches to health and safety.

The first approach is a reluctance to accept that a hazard exists. The asbestos industry still claims that there is no risk to the general public from asbestos (remarks of Alex A. Cross, chairman, standing committee, International Asbestos Information Conference at Asbestos Information Association Third Annual Industry-Government Conference, September 8-9, 1976). The second approach is to accept that a hazard does exist but that it is small. This leads to the notion of acceptable risk and threshold limit values (*Hazards Bulletin* 7, July 1977) in an effort to quantify the argument. In the case of asbestos, standards are set at 2 fibres per cc, which is a factor of ten greater than that demanded by the trade unions representing the majority of workers exposed to asbestos. Our evaluation of the literature leads us to give unqualified support to this very minimal trade union demand and we have urged trade unions to ban asbestos and get it replaced with the numerous safer alternatives that are commercially available (*The Asbestos Hazard*, Birmingham Hazards Group, 67 Woodstock Road, Birmingham 13).

A third argument employed when faced with an apparent contradiction between production and safety is to compare the risk with other already existing dangers. The use of fatal accident frequency rates (FAFR) frames this approach. According to FAFR statistics, which ignore occupational disease, non-fatal crippling accidents, the effects of shift work and other debilitating hazards, mining is less hazardous than driving a car. Such an argument paints a picture of the public as being irrational in opposing one hazard while seeming to accept another without protest. The *Nature* leader (19 May) supports this argument without recognising that there is no mechanism for car drivers to affect their hazard directly. The sensation

generated by Ralph Nader's book, *Unsafe at Any Speed*, shows how much interest there is in automobile safety and at the same time how difficult it is to mount a campaign for it. The crucial difference between these 'widely accepted' hazards and hazards at work is that people at work have the organisation, numbers, and power to effect change. The use of this inappropriate comparison serves to blunt the force of the basic demand for adequate health and safety precautionary measures. It is shocking to realise that for the vast majority of fatal accidents at work the hazard is recognised and the safeguards are known (*Accidents in Factories*, HMSO 1971).

The fourth and most critical argument is the claim that adequate protection is simply too costly. Mr Jack Sheppard, managing director of Turner and Newhall, one of the biggest asbestos fibre processing companies in the world, testified at the Government Advisory Committee on Asbestos that the TUC demand of 0.2 fibres per cc would close the UK asbestos industry entirely. The forced choice between jobs and the environment has been one of the most effective methods of getting people to accept unsafe living and working conditions. This can only be fought effectively by supporting the health and safety struggles in other countries and trying to prevent the export of hazardous operations to countries with less stringent requirements.

The BSSRS Work Hazards Group disagrees completely with the management approach of protecting the process. First, not only do we ask if it is safe; we ask, who is it safe for? Present industrial safety considerations are confined at best to the general public and express little concern for the workforce itself. Second, we ask, who pays for the cost of safety? If government or industry decides not to pay, this does not mean that the cost vanishes. Far from it. The individual affected pays the cost in poor health, lost wages and early death. This is a shifting of cost, not the saving of money. In the Robas Report, accidents were estimated to cost the nation 0.8% of the GNP. Third, in our practical work with trade unions we support the view that no matter how much information they may have, there is still a fundamental difference in perspective between the worker on the shop floor and the requirements of management.

It is this conflict between those who control production and those who need safety, between those who assign the risks and those who are exposed to them that must be acknowledged. There is a continuing need for scientists and technologists who are prepared to acknowledge this conflict to help make available to working people the information they need to ensure their health and safety.

## EUROPE

# Harmony of practice

*The European Science Foundation's general assembly heard of progress last week in controlling genetic manipulation experiments. Chris Sherwell reports from Strasbourg*

EUROPEAN initiatives in controlling recombinant DNA research will continue in spite of the hiatus reached in the United States, where no legislation now seems likely in the coming year. The pace set by the USA hitherto has slowed considerably in recent weeks with evidence that the dangers are less than at first expected, and the bill introduced by Senator Kennedy has now been withdrawn.

European efforts, however, though largely focused on the subject of legislation, are not directed at producing harmonisation of individual countries' guidelines. The chances of doing this are now widely regarded as negligible, more because of the work each country has put into creating guidelines suited to its own circumstances than because scientists have differed in their estimates of what are conjectural risks. Moreover, in practice the decisions of the European countries' equivalent to GMAG (Britain's Genetic Manipulation Advisory Group) have proved to be very similar across a broad range of experiments.

The aim now is therefore directed at harmonisation of practice in the individual countries. The intention is that individual representatives of national genetic manipulation advisory groups should supply lists of sanctioned experiments together with the relevant containment conditions. Anomalies would be discussed, and countries out of line would be expected at least to reconsider their position. Harmonisation would thus consist in concurring at regular meetings on decisions already taken.

The meetings, which should now take place every six months, are of the liaison committee for recombinant DNA research of the European Science Foundation (ESF). This met for the first time on 15 March this year, and again on 19–20 September. As a result of the first meeting, at which various countries' practices were surveyed, a small group of legal experts and scientific advisers met on 22 June to discuss common elements for possible controlling legislation. It considered licensing of facilities, consultation and compliance with controlling bodies, and an issue which had tormented the liaison committee meeting,

that of patents.

According to the ESF annual report agreed in Strasbourg last week, the September meeting supported the legal committee's finding that researchers' interests would be best protected if national committees requested information necessary only to assess suitable safety precautions. That the subject of legal safeguards and patents is a potential problem is revealed by the British case. Here, GMAG's dual role as both a technical advisory committee and as a watchdog for the public interest has made winning the confidence of industrialists, for whom the patents question is highly relevant, especially difficult.

Elsewhere the conflict of interest implicit in this dual role is resolved by creating a small technical advisory committee which looks at individual cases in detail and then delivers suitably edited reports to the full committee. It is a solution adopted on the European level as well: the ESF liaison committee's technical advice comes from EMBO's standing advisory committee on recombinant DNA research, which consists solely of molecular biologists.

The September liaison committee meeting also agreed on certain general points—that any effective legislation should provide for notification to some responsible body, and for licensing and inspection of facilities by that body. The idea that individual projects should receive prior approval was not unanimously endorsed, but the keeping of records and the idea of legislative review did receive support.

The liaison committee also considered technical safety issues which were the basis of a questionnaire distributed on the subject following a separate and informal meeting in June. The assistance of the EMBO committee will be sought on discrepancies between decisions of national safety committees and the revised NIH guidelines. Other areas of interest include the lowering of safety measures where the DNA to be cloned is pure, the criteria of pathogenicity for purified recombinant DNA molecules, the principles and application of biological containment, and the safety measures needed for recombinant DNA research using plant and animal viruses.

Out of all this a pan-European code may or may not emerge. For the moment, the objective is practical. And it has the useful purpose of serving the mutual interests of recombinant DNA researchers and of the European Science Foundation. □

## Europe's danger

A WARNING about the future of science in Europe is contained in the annual report of the European Science Foundation (ESF), endorsed last week in Strasbourg at its third general assembly. But while the threat has not been spelled out quite so cogently before by an international scientific body, the ESF announced no comprehensive plan to help counter the danger.

The problem, a product of the mid-1970s, was summed up by Sir Brian Flowers, re-elected to the ESF presidency for another three years. If the trend of dwindling support for the important innovative research done by Europe's small scientific groups continued for too long, he told a press conference, the progress of science would be affected at the point of origin. But he confessed: "This subject was not discussed very much". He added only that it was "accepted by everybody present".

Sir Brian went on to list the assembly's achievements. Apart from moves in the field of recombinant DNA (see accompanying story), these included the establishment of a committee to conduct a feasibility study on a synchrotron radiation facility, progress of an ad hoc group on taxonomy in the fields of zoology and botany, and an elevation in the status of ESF committees on the social sciences and the humanities.

Some concern was voiced privately last week at the number of ESF committees: delegates saw a need for more tangible action. At present there is only the European Training Programme for Brain and Behaviour Research, which awards grants for travel and training, and financial support for the mathematics and physics research programme of the Institut des Hautes Etudes Scientifiques. Nor did the ESF make any statement of its position on the issue of human rights.

The ad hoc group to study the design, costs and site of a European synchrotron radiation facility will be headed by Professor Hagedoorn of the Netherlands. Its predecessor, under Professor Maier-Leibnitz, found that research using such radiation sources, which is parasitic on high energy physics facilities, had reached a stage where the lack of control undermined progress. The hope is that work will start on a European facility in 1980, to be ready by 1985. This will be in addition to such other European projects as CERN, ILL, EMBL, EISCAT, JET and the European observatories.

The decisions concerning the social sciences and the humanities add two standing committees (the former to be chaired by Jean-Jaques Salomon) to the two in existence, which embrace the science research councils (ESRC) and the medical research councils (EMRC). But the idea of helping post-doctoral students in these two areas through fellowships and workshops—which in expanded and broadened form would obviously help counter the danger to science described in the ESF report—met an obstacle over how any such scheme would be financed. It proved to be the only spark of controversy at an otherwise routine gathering.

Chris Sherwell

## GERMANY

## Stagnation in industry?

*Werner Gries report from Bonn about statistics on research in Germany today*

ACCORDING to a review presented by the federal government to the German parliament, research expenditure in the federal republic amounted to DM25,960 million in 1976, 2.3% of the gross national product. The total number of staff employed in R&D, some 303,500, has been stagnating for three years; the number of persons employed in research in the industrial sector has actually declined. Expenditure for 1977 is estimated at DM27,300 million, up 5.3% on 1976.

The government's share of total research expenditure in Germany is about 51%, industry's share about 48%. Some 60% of research expenditure is effected through government grants in the industrial sector, where the state pays an average allowance of 20% of industry's own expenditure. This support amounted to DM3,000 million in 1976, of which DM1,200 million went to defence R&D and the rest to civilian R&D. Of government expenditure on civilian R&D, 72% is handled through the federal Ministry of Research and Technology.

In 1975, the latest year for which figures are available, 186,200 persons were employed in R&D in industry. This represents a decline of 6.5% compared with the peak in 1971 of 200,000 employees, but the decrease was in the number of assistant staff and not of research workers themselves. The number of research workers rose by 8.8% over 1971-1975, and assistant staffs were considerably reduced. The decisive factor may have been the pressure of costs in the German economy. As possessors of technical knowledge, research workers were not dismissed, while the work of the assistants was increasingly done by highly trained research workers.

Industry's research expenditure is concentrated in a few firms, as evidenced by the fact that 96% of research expenditure is effected by firms which have more than a thousand employees. Government promotion of research in industry has also concentrated on those firms which have the largest research budgets. Only 6.3% of government research funds in recent years have been for the benefit of small and medium-sized firms. The promotion of research by fiscal means is at present of only minor importance in the industrial sector, because several tax concessions for research were stopped in 1975. The

proportion of funds accruing to firms via fiscal promotion of research is about 2% of the total research expenditure financed by the firms themselves.

The distribution of research expenditure among the branches of German industry bears examination. Steel construction, mechanical engineering and the motor vehicle industry used 34% of the total for all firms. These industries also received 48% of government research money for industry. The number of research personnel in these industries declined in the years 1971-73, however, from 62,100 to 54,300.

The electrical engineering, precision engineering and optical industries used 30% of research expenditure by firms. They received, on average, government allowances of 15% of their own expenditure. Research staffs in these industries have increased slightly in recent years, but electrical engineering experienced a decline from about 53,900 in 1971 to 52,700 in 1973.

The third largest and most research-intensive branch of industry in Germany is the chemical, oil and plastics sector. Some 28% of firms' research expenditure is done in this branch of industry, and it receives only 2% of its research expenditure from the state. Research staffs in the chemical industry fell from a peak of 50,400 in 1971 to 47,400 in 1973. The reduction was not in the number of research workers but in their assistant staffs.

Remaining branches of industry use only 7% of research expenditure by firms. But taken as a whole the figures indicate that stagnation in research expenditure has set in even in Germany. In the industrial sector there is a real decline in research expenditure and a reduction in personnel. It is a trend which can be observed in several other western industrial countries, except for Japan.

● The federal government will distribute DM436.3 million for projects in non-nuclear energy research in 1978, chiefly to build demonstration projects in coal refining, district heating and hot water supply from solar energy. Fundamental research will be supported in coal technology, energy conversion and the efficient use of energy.

Under a special economic programme, a number of prototype plants using new energy technology have been promoted, notably the use of heat pumps in public installations. The largest (4.65MW) gas heat pump in the world so far started working at Paderborn on 21 October 1977. □

## USSR

## Brezhnev's surprise

*Vera Rich reports on Mr Brezhnev's jubilee speech for the sixtieth anniversary of the October revolution*

MR BREZHNEV's jubilee speech followed traditional lines for the most part but contained two surprises. The most remarkable feature of his speech was the section on reducing the danger of nuclear war. In addition to the well-reported commitment to an eventual total ban on nuclear weapon tests—to include underground as well as atmospheric, outer-space and underwater tests—he stated that the Soviet Union was prepared to agree to a moratorium on peaceful nuclear explosions (PNE).

PNE forms an important part in the Soviet plans for diverting the Siberian rivers southward to irrigate the arid steppes of Central Asia. This diversion is not entirely dependent on PNE: for explosions below 10 megatons, conventional explosives are more cost-effective, except where difficult terrain poses special logistic problems. Nevertheless, a year ago the Soviet media began to comment extensively on the use of PNE in the project, and it was envisaged as a major mark of progress. Indeed, so anxious were the Soviet authorities to use PNE in the diversion project that they were even prepared to agree to on-site American inspection. Now, however, Mr Brezhnev is apparently willing to sacrifice the whole project as the price of a test-ban treaty, and to revert to more conventional means of canal-building.

The other major surprise was the figures for this year's harvest. In spite of fervent denials by the foreign services of Moscow radio, Western experts had expected a shortfall of some 8 million tonnes. Although Mr Brezhnev made no reference to shortfall, the total yield of 194 million tonnes means that the shortfall from the target is no less than 19 million tonnes. Although the weather must be largely held responsible, it is not surprising that the future plans for agriculture stress the intensive development of agriculture.

Other present and future plans outlined in the speech include the opening up of Siberia by the Baikal-Amur railway, the new plans for developing the non-black earth zone, and "the discovery of new sources of energy and substitutes for many types of natural resources, the technical re-equipment of the economy to reduce manual and especially arduous labour to a minimum, the boosting of agriculture, the combating of disease, and the prolongation of the human life-span". □



## IN BRIEF

**Porton decision due**

A decision on the future of the Microbiological Research Establishment at Porton is expected shortly now that the Medical Research Council has considered the complete report of its special advisory committee under Professor Sir Robert Williams and transmitted it to the Secretary of State for Education and Science. The Ministry of Defence is due to leave the establishment by 1 April next year.

The advisory committee assessed the scientific potential of Porton for civilian research. The Institute of Biology has published its written evidence in the latest *Biologist*. The institute considers that the pilot fermentation plant, enzyme laboratory and containment facilities, together with staff and ex-

pertise, should be retained, developed and exploited rather than dissipated and dispersed.

**Nuffield's good turn**

The Nuffield Foundation has decided to help UK scientific and medical research, by supporting projects which would normally receive research councils' money but which might go unfunded. It means a temporary change of policy for the Foundation, which was set up to support research leading to social benefit for which there is no ready source of public money. The Trustees have already allocated funds amounting to about £60,000 for research which they normally would not consider. The level of support is expected to increase next year.

In 1976, of the £1.67 million income (up almost 25%), £1.16 million was spent on support for research. Only £50,000 of that went on scientific research, however, and £70,000 on medical research. The latest annual report says one reason for the small demand on Foundation resources may have been that researchers assumed funds were low after the Foundation sold its shares in British Leyland.

**NASA reorganisation**

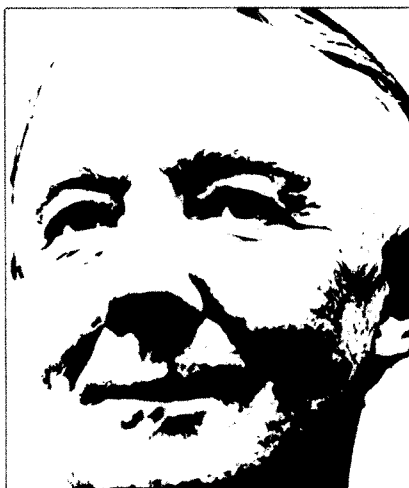
A reorganisation of NASA by its administrator, Robert Frosch, includes a revised role and title of chief scientist, who will advise on the agency's "total programme from the standpoint of scientific objectives". The change takes effect this week.

Most people think that the only way to conserve wild plants and animals is to leave them alone. They imagine that the perfect nature reserve is an area surrounded by a man-proof fence, where natural processes can proceed without his baleful influence. This is sometimes true. The immense national parks in North America, areas like the Kruger Park in South Africa, and some reserves in the less-inhabited parts of Poland and Scandinavia require the minimum management of their vegetation, though even under these favourable conditions the larger mammals may increase beyond their optimum and require control.

The situation in countries like Britain is generally very different. We have a few large nature reserves in the highlands and islands of Scotland where nature can be left to take care of itself. Here the main management is to control man, including over-zealous naturalists who wish to collect rarities, and over-enthusiastic ecologists whose scientific sampling may destroy the vegetation. But even the most 'natural' area has probably been modified by human activity, perhaps by eliminating wolves and other predators, which allow deer to increase to numbers which ruin the vegetation unless they are severely culled.

In most areas man has reduced the tree cover and so encouraged the development of moorland vegetation. The conservationists, many of whom believe they are preserving natural conditions, oppose any move by farmers to restore the fertility destroyed by the agricultural techniques of our iron age ancestors. They produced the present beautiful—but semi-desert—landscape of, for instance, Exmoor.

Nevertheless, in many parts of Britain the native vegetation may develop if it gets the chance. The famous Broadbalk Wilderness at Rothamsted Experimental Station was a wheat field until it was enclosed and

**Managing wildlife****KENNETH MELLANBY**

left alone over a hundred years ago. Soon shrubs and trees appeared, and it is now a small wood of oak, ash and other indigenous species. At Monks Wood in Huntingdonshire we started a similar experiment on a larger scale in 1960, by leaving the ten acre Stocking Close field, an area which had been arable for centuries, uncultivated. Within seven years it was covered with a scrubby growth of hawthorn and dog rose, with a scattering of oak of up to 100 saplings per acre. It was clearly on the way back to something very like the natural deciduous woodland which covered southern Britain before man

cleared it for his farms.

However, such natural regeneration may be thwarted by some of the exotic species man has introduced to Britain. When we began the Stocking Close experiment, rabbits were very scarce following the spread of myxomatosis in 1955. Today rabbits are common again, and though acorns are still scattered around the countryside by jays, pigeons and small mammals, many seedlings are destroyed. In some parts of the country the aggressive imported sycamore prevents native tree species from becoming dominant, and so management is needed to produce natural forest.

Things may be even worse in other countries, particularly in oceanic islands. In Mauritius, for instance, conservation is a nightmare. Man has been there for under 500 years, and in any numbers for 200, but he has left little of the unique indigenous flora and fauna. Though he has exterminated birds like the dodo, and removed most of the natural vegetation to grow sugar cane, it is his introductions, plants and animals, which provide the worst problems today. Where small relict areas of indigenous trees are preserved, they are swamped with the rampant growth of guava and bramble (introduced species) producing impenetrable thickets which defy control. The introduced monkeys delight in destroying the nests of the rarest birds, and mongooses in killing the most interesting mammals and reptiles. Thus conservation may provide the most difficult management problems not in developed areas where man is obviously dominant, but in an oceanic paradise where nature might be expected to control the situation.

# correspondence

## What is unusual?

SIR,—In Sakurai's interesting discussion of the relation between equatorial solar rotation and climatic changes (29 September, page 401), a rather unfortunate choice of terminology is used in referring to "the Earth's present unusual climatic conditions". Unusual by what standard? The best evidence of available data covering the past 1,000 years or so suggests that the period roughly from the 1920s to the 1960s was the most unusual 50-year period of the entire millennium, in climatic terms, at least for northwest Europe (H. H. Lamb, *Climate: Present Past and Future*, vol 2 (Methuen, 1977)). Studies such as that of Eddy (*Scient. Am.* **230**, 80–92, 1977) indicate that this was also an unusual period in sunspot terms, with very high peak sunspot numbers, in line with the general conclusions of Sakurai, Volland (28 September, page 400) and others.

The possibility of a return of climate towards nineteenth century conditions should not be regarded as 'unusual' climate except on the scale of a human lifetime; the unfortunate coincidence that an explosive growth of population and demand for food has happened over just that time makes it all the more important for us to develop an understanding of what really is 'normal' in climatic terms, and I would therefore urge Sakurai and other workers in this field to avoid misleading use of emotive terms such as "unusual". If the relevant sentence is rephrased to say "variation of the equatorial rotation speed may be responsible for the recent unusual climatic conditions on Earth, now returning to the normal conditions of recent centuries" the implications of the change for the activities of mankind are much more clearly apparent.

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## Missions to Halley's comet

SIR,—The informative article by David Hughes (11 August, page 468) concerning the proposed fly-by and rendezvous missions to Halley's Comet was, unfortunately, less than fair to the achievements of those who have been striving to perfect various space electric propulsion techniques.

In particular, his description of ion drive and solar-sail propulsion as "... two mechanisms (that) are still at the theoretical stage ..." is certainly far from true of ion propulsion systems.

Several laboratories in the USA and in Europe have devoted many years of effort to developing ion thrusters of different kinds. The electron bombardment ionisation type, originally devised by Kaufman, has emerged as firm favourite, at least in the USA and the UK, and several versions are now ready for space qualification. These include the 10 cm diameter, 10 mN thrust T5 device developed by the Royal Aircraft Establishment, with assistance from the UKAEA Culham Laboratory and British industry, and two thrusters of 8 and 30 cm diameter, produced by Hughes Research Laboratories under a programme directed by the NASA Lewis Research Center.

The highly-efficient 30 cm thruster is particularly well-suited to cometary rendezvous missions. Its thrust of about 130 mN is appropriate to a multiple thruster, modular propulsion unit concept, with power being derived from solar arrays. This principle readily allows a wide range of throttling to match the available power as the distance of the spacecraft from the sun varies.

The application of this thruster to cometary missions has already been studied in great depth, particularly with regard to Comet Encke, and detailed designs have been produced of the necessary flight systems.

It should also be pointed out that, far from being "at the theoretical stage", two Kaufman-type ion thrusters were flown experimentally as early as 1970. They then operated successfully for 3,763 h and 2,011 h. In 1976, one was still capable of being run under its design conditions, whilst the other exhibited a single fault.

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## Engineers' salaries

SIR,—In your editorial 'Room at the top—for whom?' (22 September, page 275), you quoted, from our report to the British Association Coordinating Group, some figures concerning salaries of engineers compared to other pro-

fessional groups in manufacturing industry. The inference you draw, that salaries in production industry "... discriminate against the scientist and engineer ...", is exactly what the graphs of median age/earnings profiles appear to show, but the point we wished to bring out was that this inference is not justified.

The graphs were of median and upper quartile salaries by age for all the professional staff (22,400) in a number of leading companies. Within this total, there were 7,386 engineers compared to 607 chartered accountants and only 141 lawyers, and we were careful to give the number in each professional group. Chartered accountants are trained, and in general gain their early experience, outside industry. A limited number are recruited by industry for functions which of their nature are mainly concerned with matters at or close to Board level. By contrast, as we show elsewhere in the report, the majority of graduate engineers initially go into industry, and industry also has an overwhelming stake in those who qualify via HNC/HND and institution membership. They are employed in a wide range of jobs, from basic technical work to Board level.

Therefore, if industry succeeds in its normal job evaluation objective, to pay comparable salaries for comparable jobs, small groups of professionals selectively employed by industry will show higher median or upper quartile age/earnings profiles than a large group of professionals generally employed. It is tempting to believe that upper quartile, and in particular upper decile, graphs will show a fair comparison, but unless the samples are carefully matched in each age group this is not so.

Using exactly the same salary data, we went on to look at the number in each professional group earning salaries on or above £10,000 a year. This is one way for the companies in the survey, of answering your headline question "Room at the top—for whom?". The 749 engineers enjoying these salaries represented only 10.1% of their group, but they outnumbered the accountants, lawyers, economists and arts graduates put together.

VINCENT EDKINS

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# news and views

## Tubulin assembly

from L. A. Amos

THE factors controlling the assembly of tubulin into microtubules are still under intensive study, although it is now five years since Weisenberg (*Science* **177**, 1104; 1972) first found conditions in which microtubules would assemble from a brain protein extract at 37 °C and disassemble on cooling to 5 °C. Microtubule protein purified by several cycles of assembly and disassembly does not consist only of tubulin: a number of so-called microtubule-associated proteins are observed to copurify with tubulin, most of whose functions are as yet unknown.

In 1975, it was reported that tubulin purified by ion exchange chromatography would not reassemble under Weisenberg's conditions, unless at least some of the associated proteins were returned to the mixture (Weingarten, *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 1858; 1975; Murphy & Borisy *Proc. natn. Acad. Sci. U.S.A.* **72**, 2696; 1975). The accessory factors in the extract were shown to be protein and seemed to act stoichiometrically rather than catalytically in stimulating tubulin assembly. Much of the controversy since has arisen because of the considerable variation in non-tubulin components of microtubule protein purified in slightly different conditions in different laboratories. In many cases, the most prominent components appear on SDS polyacrylamide gels as a small group of bands corresponding to unusually high molecular weights (of the order of 300,000 daltons) which have been called HMW (Borisy *et al. Fedn. Proc.* **33**, 167; 1974). Other less prominent bands are observed between 55,000 and 80,000 daltons. Borisy *et al.* found HMW in stoichiometric amounts in their preparations, corresponding to one HMW molecule per 10–15 tubulin dimers, but much lower proportions of HMW to tubulin have been reported. The positions and intensities of the other non-tubulin bands seem also to vary. In preparations from Kirschner's laboratory, HMW is found only as a minor component. He therefore suggested that the assembly-stimulating

factor, which he named 'tau' was one of the lower molecular weight proteins.

Recent papers by Murphy *et al.* (*Biochemistry* **16**, 2598; 1977) and Cleveland *et al.* (*J. molec. biol.* **116**, 207; 1977) both describe the fractionation of the associated proteins into HMW and tau components. Although the two groups used quite different methods of chromatography to effect the separation, the results are in good agreement. Both found the activity present in both major fractions. Murphy *et al.* ascribe 60% of the activity to HMW and 40% to the tau fraction. Cleveland *et al.* found a third of the original activity in their tau fraction, the remainder presumably being in the HMW fraction, which in their preparations also included some very low molecular weight material.

Cleveland *et al.* have gone on to purify further the active proteins in the tau fraction and have identified a family of four polypeptides with molecular weights between 55,000 and 62,000 which they name tau I–IV. In an accompanying paper (*J. molec. Biol.* **116**, 227; 1977) these proteins are characterised in some detail and are shown to have some interesting properties, including the ability to retain their activity after being heated to 75 °C for 1 h. By comparison, the activity in the HMW fraction is much less stable. Although the tau molecules seem to be highly extended, the amino acid compositions (including relatively high proportions of glycine and proline) and circular dichroism measurements suggest a low content (12%) of  $\alpha$ -helix. Finally, their overall charge is neutral or only slightly basic.

The latter property is rather surprising in view of the assembly facilitating activity shown by various unrelated polycationic substances, such as poly-L-lysine, DEAE dextran and RNase A (see Erickson & Voter *Proc. natn. Acad. Sci. U.S.A.* **73**, 2813; 1976), although the polymers formed in such cases are not usually normal microtubules. It has also been shown recently (Herzog & Weber *Proc. natn. Acad. Sci. U.S.A.* **74**, 1860; 1977) that pure tubulin can be induced to assemble into microtubules simply by the addi-

tion of 10 mM MgCl<sub>2</sub> to Weisenberg's buffer. However the critical tubulin concentration is 10 times as high (2.5 mg ml<sup>-1</sup>) as in the presence of associated proteins and the resulting microtubules are considerably less stable. As Cleveland *et al.* point out, the role of the accessory proteins in assembly seems to be to shift the equilibrium for an interaction which is unfavourable under normal conditions. The manner in which this is achieved does not seem to be simply a matter of counterbalancing charges on the tubulin molecules.

The possible relationship between tau and HMW is likely to be debated for some time. When it was discovered that HMW is readily proteolysed, even during storage of the purified protein, Sloboda *et al.* (*Biochemistry* **15**, 4497; 1976) suggested that tau might consist of proteolysed fragments of HMW. From comparisons of amino acid composition and one-dimensional peptide mapping, Cleveland *et al.* conclude that tau I–IV are closely related to each other but not to the HMW material in their preparations. However, the results presented so far may not be sufficient to convince everyone; since a tau molecule derived from a specific region of HMW would correspond to only 20% of the original molecule, such a relationship would not necessarily be obvious from the present experiments.

One of the more interesting cases of abnormal assembly of tubulin is the formation, from unfractionated microtubule protein, of extended sheets, induced by low levels (0.25–1.0 mM) of zinc. This effect was first reported by Larsson *et al.* (*Expl Cell Res.* **100**, 104; 1976). The sheets offer an opportunity of observing the structure of an extended crystalline array of tubulin molecules by electron microscopy and applying image analysis techniques, including optical diffraction and computerised image reconstruction. Crepeau *et al.* (*J. molec. Biol.* **116**, 301; 1977) have carried out some preliminary studies in this direction, and have compared the structures of Zn-induced sheets and the smaller tubulin sheets formed under normal buffer conditions during microtubule assembly. In the

Zn-induced sheets, the relative arrangement of tubulin protofilaments appears to be different from that in the 'normal' sheets and in microtubules, but the protofilaments seem to be essentially the same.

The results show the structure in more detail than has been observed before, although the present resolution is still only about 20Å. Some preliminary studies on unstained sheets suggest that it may be possible eventually to achieve the sort of high resolution results obtained by Henderson and Unwin in their study by electron microscopy of the purple membrane from *Halobacterium* (*Nature* **257**, 28; 1975). □

## Primate social structure and ecology

from John Krebs

GIBBONS (*Hylobates* spp.) live in pairs and defend a territory of less than 1 square kilometre; baboons (*Papio* spp.) wander over an undefended home range of 15–20 square kilometres in polygamous or promiscuous groups of about 40 individuals. These contrasts in group size, home range, mating system and so on are typical of the bewildering array of social organisations in primates, and one of the major aims of primate ethologists over the past 15 years or so has been to seek regular patterns in the differences between species.

J. H. Crook and J. S. Gartlan (*Nature* **210**, 1200; 1966) made the first serious attempt to link primate social structure with a small number of ecological variables such as food dispersion and pressure from predators. They noted, to take just one example, that ground-living primates such as baboons live in larger groups than their arboreal relatives and suggested that this could be an antipredator adaptation, terrestrial monkeys being more exposed to predators and hence benefiting more from communal defence in a large group. Crook and Gartlan clearly had the right approach, but their attempt to classify primate societies into about half a dozen ecological categories foundered because as more field evidence came to hand, too many exceptions to their rules emerged. This led to two modifications of the original approach. Some argued that Crook and Gartlan were wrong to assume that primate social organisation is always adapted to present day conditions, and that phylogenetic heri-

## Nitrogen diffusion in diamond

from John Walker

THE results on conversion of diamonds from type Ib to type Ia which are reported in this issue of *Nature* by Chrenko, Tuft and Strong (page 141) have important implications for diamond physics and for synthesis of gemstones.

The Platonically ideal diamond is composed only of carbon. Real diamonds, however, contain impurities, and paradoxically, the large, regularly shaped and apparently perfect gemstones are usually the most impure. (This effect is well-known to crystal-growers—impurities affect the shape of many types of crystal.) In diamond the most important impurity is nitrogen. A diamond is classified as type I if it is appreciably impure, and as type II if it is relatively nitrogen-free. The nitrogen may be present as isolated individual atoms (type Ib), giving the diamond a yellow colour, a paramagnetic resonance signal and a characteristic infrared absorption; or it may be in aggregates, especially pairs (type Ia), resulting in no paramagnetism, no visible colour and a different infrared spectrum. This type-classification is qualitative, and diamonds of mixed type are often found, but it is useful nonetheless.

The vast majority of natural diamonds are type Ia, but synthetic crystals are usually type Ib. Nobody knows why for sure; a plausible hypothesis is that some natural diamonds at least, started life as type Ib, and were subsequently transformed to the Ia type.

Diamond is the metastable form of carbon at normal temperature and pressure. It is stable, and so can be synthesised, at higher temperature and pressure. The hypothesis is that, in some conditions, although nitrogen can be incorporated into the growing crystal, it is immobile; subsequent higher temperatures or lower pres-

ures allow it to become mobile and diffuse through the carbon lattice until it can aggregate with other nitrogen atoms. Chrenko *et al.* report that after annealing at 2,000 K and 60,000 atmospheres for 30 min the type Ib diamonds had become appreciably paler yellow in colour, due to reduction of their characteristic visible absorption, and that their paramagnetism was weaker. The infrared spectrum had changed from typical type Ib to mixed Ia and Ib, and the N3 ultraviolet absorption system (thought to be an aggregate of three nitrogen atoms) could be detected. The total nitrogen concentration remained constant—a useful consistency check.

What is the significance of these results? First, they tell us more about how nitrogen behaves in diamond; and they may help us to understand other defects, especially the 'platelets'—laminar defects on {100} planes which have intrigued and puzzled diamond physicists for 40 years. Second, they indicate a method of turning yellow synthetic diamonds into 'water-white' ones, thus enhancing their value as gemstones. Third, they tell us more about the synthesis process, which may lead to the commercial synthesis of large gem-quality diamonds. (At present, although tons of synthetic diamonds are produced annually, they are suitable only for industrial applications—it is actually cheaper to dig the gemstones out of the ground than it is to synthesise them.) Finally, the results may have implications for the conditions prevailing in the Earth's mantle that led to the natural synthesis of diamonds. As Sir Charles Frank put it, 'Nakaya has written a poem in which he says, "A snowflake is a letter to us from the sky." A diamond is a letter to us from the depths, and a letter more worth reading because we can visit the sky.'

tage plays a strong role (Struhsaker *Folia Primat.* **11**, 80; 1969) while others proposed that Crook and Gartlan were in principle right, but that the differences in ecological pressures had to be analysed on a much finer scale (Clutton-Brock *Nature* **250**, 539; 1974), and further that the same ecological pressure may lead to different solutions.

In an important new review, T. H. Clutton-Brock and P. H. Harvey (*J. Zool. Lond.* **183**, 1; 1977) have greatly extended and updated Crook

and Gartlan's original approach. They have classified all the primate species for which they could find data into nocturnal or diurnal, arboreal or terrestrial, and insectivorous, frugivorous or folivorous. They then did a quantitative statistical analysis to look for consistent differences between these ecological categories in features such as group size, home range, sex ratio and body weight. The main trends to emerge from their analyses were that nocturnal primates which are all arboreal and mainly eat either

fruit or insects, are small, live in small groups and have small home ranges; both diurnal and nocturnal fruit eaters have large bodies, and live in larger groups and home ranges than leaf eaters; and finally, diurnal terrestrial primates live in larger groups and ranges and have more marked sexual dimorphism than do diurnal arboreal species.

How are these general trends related to ecological needs such as food gathering and avoiding predators? Clutton-Brock and Harvey suggest that the nocturnal species have to have a small body because they feed by crawling on to small branches, and they rely on crypsis to escape from predators. At the other extreme, conspicuous terrestrial monkeys living in open habitats have evolved large body size partly as an antipredator device, and partly to allow them enough mobility to search over a wide area for scattered food. Although Clutton-Brock and Harvey agree with Crook and Gartlan's idea that large groups in terrestrial primates may be an antipredator adaptation, they also point out that there are large differences in group size within both the arboreal and terrestrial categories. They propose that larger groups are always advantageous in diurnal primates, perhaps as an antipredator device, but that competition for food sets an upper limit to group size, so that species feeding on large clumps of food can afford to live in larger groups than those eating more evenly scattered food. A comparison of two types of *Colobus* monkey, one of which eats fruit, flowers and shoots (highly clumped food), and the other which eats leaves of all ages (less clumped) supports the idea: the former live in larger groups.

Home range size is obviously closely related to group size and is probably determined both by the minimum area needed to support the group, and by the density and dispersion of food. Species feeding on evenly dispersed, fairly dense food supplies have small, often defended home ranges, while those eating widely scattered clumps of food tend to have large undefended ranges, for obvious economic reasons.

So far, the differences in social structure seem to be well explained by straightforward ecological pressures, but it is not so easy to account for the variations in mating system and adult sex ratio. Monogamy is very rare in primates, and it must presumably have evolved only when a male significantly increases his output of young by helping the female (for example, monogamous marmosets have twins and the male helps to carry them), or because the male cannot secure more than one

mate. (This may seem an unduly chauvinistic view, but the sad fact is that female mammals as a whole have little choice about who does the parental care.) The second of these possibilities may account for monogamy in species such as the gibbon in which the even distribution of food favours territorial defence, and males may be unable to defend enough food to support more than one wife. The majority of primates breed in harem or multimale groups, and the ecological factors favouring one system over the other (and the general bias of adult sex ratio towards females) are not at all clear. It is, however, apparent that strong sexual dimorphism ( $\sigma : \text{weight ratio}$ ) is related to the degree of bias in the sex ratio and to polygamous or promiscuous mating. This suggests that sexual dimorphism in primates has evolved when males compete for the chance to mate.

Clutton-Brock's and Harvey's review emphasises the fact that it is possible to account for many of the differences in primate social structure by pressures associated with detailed dispersion of food and vulnerability to predators. It also reveals that the various dimensions of social organisation, such as group size, sex ratio and so on, may be influenced by different ecological factors and should therefore be analysed separately. One of the next steps will be to see to what extent variations within a species in different habitats can be used to test the ideas generated by interspecies comparisons.

## Biochemical oscillators

from N. MacDonald

OSCILLATORY phenomena in organisms, with periods of seconds or minutes, are known in great variety. The most obvious example is the heart pacemaker oscillation in the membrane potential of the sino-atrial node. Oscillatory membrane potentials also occur in neurones, in cells secreting hormones, and in smooth muscle. The interpretation of such phenomena in terms of underlying oscillatory biochemical reactions presents a challenge to both experimentalists and mathematicians. Identification of the steps in a feedback loop is not sufficient, unless the likelihood of instability of the steady state is established. Formidable

difficulties arise from the nonlinear dynamics of enzyme-catalysed reactions, from the length of the chains of reactions in feedback loops, and from the linking of these loops in complex networks.

In seeking to reduce networks of reaction equations to manageable proportions one relies heavily on the fact that the time scales for biochemical reactions vary widely. Retaining for detailed study those reactions with relaxation times rather smaller than the period of the oscillation, the other reactions may be subsumed into parameters. Particular interest then attaches to the values of these parameters at which qualitative changes occur in the nature of the solutions of the equations retained. In mathematical terms, this is the field of bifurcation theory. Alternatively one may retain more equations, but classify them into fast and slow sets. Then it is necessary to explore situations in which smooth changes in the slow dynamics lead to qualitative changes in the nature of the fast dynamics. This is the field of catastrophe theory, as discussed in this context in the recent review by Heinrich, Rapaport and Rapaport (*Prog. Biophys. molec. Biol.* **32**, 1; 1977).

Both the review by Heinrich *et al.* and that by Goldbeter and Nicolis (*Prog. theor. Biol.* **4**, 66; 1976) are largely concerned with detailed models of oscillations in the glycolytic pathway. Here direct evidence of periodicity in the concentrations of chemical species is available, and a great deal of work has been done in constructing and testing models. The models examined in these reviews stress the activation and inhibition of the enzyme phosphofructokinase by one or more of the adenosine phosphates AMP, ADP and ATP.

Much progress has been made recently in the mathematical analysis of the single loop, end-product inhibited reaction sequence advocated by Goodwin (*Adv. in Enzyme Regulation* **3**, 425; 1965) as a paradigm for a biochemical oscillator. One highlight in this is the theorem of Hastings, Tyson and Webster (*J. Differential Equations* **25**, 39; 1977) which is applicable to a wide class of such loops, and allows nonlinearity in all the equations. This theorem states that instability of the steady state solution is a sufficient condition for the existence of at least one periodic solution, although unfortunately this solution need not be stable. Another highlight is the systematic application of approximate methods by Rapp and Mees (*Math. Biosciences* **25**, 165; 1975; *J. math. Biol.* **3**, 203; 1976 and in the press) to estimate periods and amplitudes of these solutions.

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Factors tending to promote instability of the equilibrium point, and the onset of the attendant oscillations, are of three kinds. Strong nonlinearities, such as can be expected with allosteric enzymes, help. So do the presence of a large number of successive reactions in a loop, and the similarity of the time scales of all these reactions. These two factors go together, since widely different time scales allow some of the reactions effectively to be ignored. There is still a wide gap between the appealing idea of the end-product inhibited oscillating loop, now put on a firm mathematical basis, and the presentation of a fully worked out and experimentally testable scheme, possessing these factors, for some specific oscillator. End-product inhibition is rife in cell chemistry, but this can be understood solely in terms of advantageous properties of steady states. In a well-defined sense (Savageau *Biochemical Systems Analysis*, Addison Wesley, 1976) these states have minimal, which implies optimal, sensitivity to parameter change.

A bold attempt has recently been made by Rapp and Berridge (*J. theor. Biol.* **66**, 497; 1977) to implicate, in a wide range of oscillations, end-product inhibited loops involving the binding of calcium ions. They propose two different styles of loop, involving calcium in conjunction with cyclic AMP. In the first an increase of cyclic AMP causes an increase in cytoplasmic calcium, either by promoting calcium entry through the cell membrane, or by causing a shift in cell calcium from a bound state to the cytoplasm. Cytoplasmic calcium on the other hand is taken to inhibit the synthesis of cyclic AMP, acting through the inhibition of the allosteric enzyme adenylyl cyclase. The alternative loop reverses the activating and inhibiting roles of cyclic AMP and calcium.

Sustained oscillations have been observed in the transepithelial potential of the salivary gland of the blowfly *Calliphora*. Rapp and Berridge cite evidence that here cyclic AMP can release calcium from some intracellular store, and that this store may be in mitochondria. They also cite evidence that calcium can inhibit cyclic AMP synthesis in this gland, and that this may be by inhibition of adenylyl cyclase, for which there is evidence in certain cells of a variety of organisms. On the other hand, they argue that the second kind of loop may be responsible for the periodic contractions of smooth muscle. They also examine cardiac pacemaker oscillations, membrane potential oscillations in cells producing insulin, and signalling in the slime mould *Dictyostelium*.

In all these contexts their evidence is

cumulative, seeking to implicate calcium as significant in contexts in which oscillations occur rather than proposing detailed dynamics. It is a somewhat daunting thought that each context may require a similar amount of effort to that devoted to glycolytic oscillations over the years. But the work of Rapp and Berridge should focus critical attention on a particular set of possible common mechanisms for a variety of periodic phenomena. □

## Acceleration of auroral particles

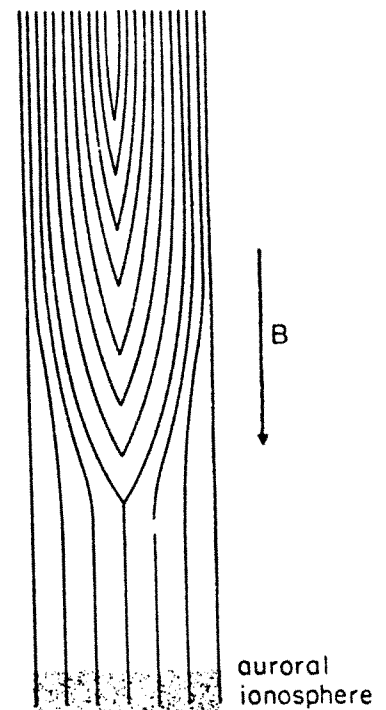
from Alan Johnstone

AURORAL electrons are accelerated into the upper atmosphere by electric fields parallel to the magnetic field lines with the total potential difference sometimes exceeding 10 kV. The fact that the acceleration is caused by a quasi-static electric field, and the magnitude of the potential difference can be deduced from measurements of the angular and energy distributions of the precipitating electrons. The electric field increases the electrons' velocity parallel to the magnetic field leaving the perpendicular component unchanged. This creates an angular distribution which peaks in the downward direction parallel to the magnetic field. This contrasts with the angular distribution of the source population of trapped electrons which usually has a minimum along the field. All electrons are given the same increase in energy ( $e$  times the potential drop) so that any observed at lower energies must have been either backscattered from the atmosphere and then reflected from the potential, or scattered out of the beam by wave-particle interactions. As there are fewer of these electrons the energy spectrum develops a peak close to the energy corresponding to the potential differences. Sounding rockets and satellites have found that such electron distributions are common above auroral arcs. The acceleration is found to be greater in the centre of the arc than on the northern and southern borders implying that the equipotential surfaces in a meridional slice above the arc are V-shaped as in Fig. 1.

The accelerated electrons have been detected at altitudes up to 1,500 km and ions accelerated in the opposite

direction have been detected by Geostationary satellites near the equator, but the altitude where the particles were accelerated could not be located until the S3-3 satellite was put into an elliptical polar orbit with apogee at 8,050 km and perigee at 260 km in 1976. In a year of operation it has traversed the auroral zone at all altitudes between these extremes. Convincing evidence from this satellite that particles are usually accelerated somewhere in the range between 2,000 km and 7,000 km was one of the highlights of the recent IAGA meeting in Seattle. P. Mizera and J. Fennell (Aerospace Corp.) showed examples of upward-moving, magnetic-field-aligned ion beams accelerated below the satellite by several kiloelectron volts detected simultaneously with downward-moving electron beams accelerated by comparable amounts above the satellite (Fig. 2). This is a clear indication that the satellite is in the middle of the acceleration region. The ion beams include both  $O^+$  and  $H^+$  ions (Shelley *et al. Geophys. Res. Lett.* **3**, 654; 1977), confirming that they originate in the low-altitude ionosphere. The probability of observing an accelerated ion beam in a traversal of the auroral oval is as high as 95% at altitudes above 7,000 km and less than 5% below 2,000 km. What the data do not tell us is how the electric field is

**Fig. 1** A schematic diagram of the equipotential contours above an auroral arc. The innermost contours are negative so that electrons are accelerated downward parallel to the magnetic field  $B$  into the auroral ionosphere (Swift *et al. J. geophys. Res.* **81**, 3931; 1976).



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distributed in this height range. It could all be confined within a narrow layer, as some theories require, or it could be distributed evenly over the entire length. If the layers are thin then at least two must exist on the same field line in order to give the simultaneous observation of upward accelerated ions and downward accelerated electrons. F. Mozer (Berkeley) presented measurements of the ambient electric field from the same satellite (see also *Phys. Rev. Lett.* **38**, 292; 1977). The magnitude of the electric field, in structures which he termed 'paired electrostatic shocks', was more than  $0.5 \text{ V m}^{-1}$  perpendicular to the magnetic field with a smaller parallel component. This is far greater than the field strengths found below 1,000 km. The structure in the paired electrostatic shock is just the same as would be found traversing the V-shaped potential of Fig. 1 and the potential difference between the centre and the edges can reach several kilovolts if it is treated as a purely spatial variation. The accelerated ion beams and paired electrostatic shocks are found in the same region but the detailed correlation is not close. The ions spread over a region more than five times the thickness of a typical electric field structure. If they were accelerated upward by a V-shaped potential distribution beneath observed electrostatic shocks they would only be found in the centre of the structure itself. The detailed comparison of the electric field and particle observations is still at an early stage. The results of analysis now underway will be interesting and significant.

These data do not discriminate between the various proposed physical mechanisms which could be maintaining the kilovolt potential differences. Until the observational evidence for parallel electric fields became overwhelming it was thought that the plasma conductivity was too great to allow potentials of more than a few volts to develop along a magnetic field line. Strong electric currents flow upwards along magnetic field lines above auroral arcs (carried by a downward flux of electrons) which may exceed the current density the plasma can support with low resistance. Three mechanisms have been proposed to explain the formation of high potentials with strong field-aligned currents. The electrostatic shock layer, which has been shown to exist as a shock-like solution to the Poisson/Vlasov equations for strong currents, has a thickness of the order of an ion gyroradius (D. Swift, Alaska). This is related to the double-layer, also current-controlled, but not much thicker than a Debye length. Anomalous resistivity

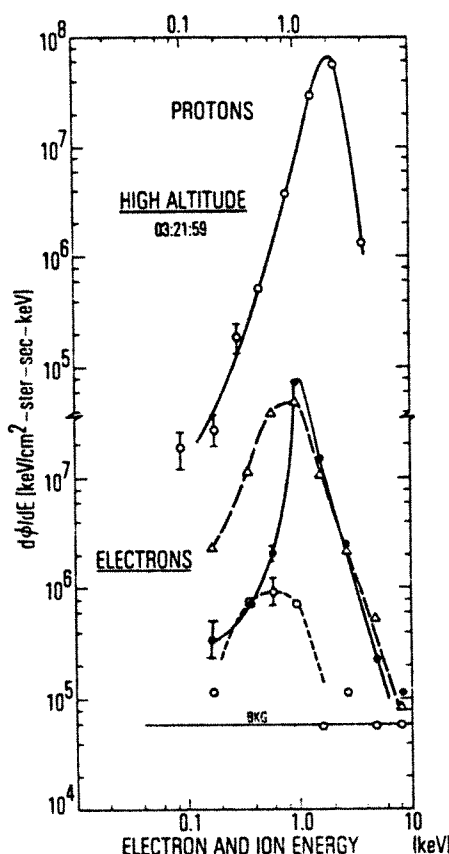


Fig. 2 Differential energy flux spectra for protons and electrons at 7,300 km altitude over the northern auroral zone. ○, Upcoming particles; ●, downgoing; △, mirroring. The mirroring electrons below the peak may be trapped between the converging magnetic field on one side and the electric potential on the other. (P. Mizera and J. Fennell, Aerospace Corporation).

is created when the current flow becomes unstable to the generation of electrostatic ion cyclotron waves. The resultant electrostatic turbulence hinders the electron flow. Finally, high voltages can be required to drive a current in a hot plasma down the converging field lines against the magnetic mirror force.

Evidence for all three mechanisms can be found in the S3-3 data but none of it is conclusive. C.-G. Fälthamar (RIT, Stockholm) suggested that more than one of them may be operating. For example, the plasma turbulence associated with anomalous resistivity may help the formation of a shock layer.

If it is becoming clearer how the parallel electric fields might be maintained, it is still not clear how the contours above the V-shaped layer in Fig. 1 close, and where the energy source which drives the field-aligned currents is to be found. It was noticeable at the IAGA meeting that most scientists discussing auroral acceleration mechanisms ignored those regions of the magnetosphere above the acceleration region and that those con-

cerned with auroral substorm-related events in the far plasma sheet and geomagnetic tail ignored the influence of the ionospheric ends of the magnetic lines of force. The time has come to try to fit the two ends together. □

## Microanalysis in biology and medicine

from Patrick Echlin

The Third International Conference on Microprobe Analysis in Biology and Medicine was held in Münster on 5-8 September, 1977 under the auspices of the German Electron Microscope Society and the Royal Microscopical Society. The papers will be published by Hertzl-Verlag, Stuttgart, later this year.

THE previous meetings in 1973 and 1975 showed that a wide range of microbeam analytical techniques could be used in biological investigations. The meeting in Münster demonstrated that the methods are at last providing physiologically significant data from a wide range of plant and animal tissues, which are in close agreement with similar data obtained using ion selective electrodes, flame photometry and chemical analysis.

In many instances microbeam techniques are taking analysis beyond the limits of more conventional methods, as was clearly shown in papers on the application of electron probe X-ray microanalysis to cells and tissues. T. Hall (University of Cambridge), claiming that the spatial resolution was now as low as 20 nm and the performance better than  $10^{-18} \text{ g}$  of an element, showed how X-ray microanalysis could be used for quantitative analysis of diffusible ions in frozen-hydrated sections of fluid-transporting epithelia. A. Somlyo (University of Pennsylvania) was able to show, using freeze-dried sections of smooth muscle, that the high cellular chloride in this tissue was not due to compartmentalisation in organelles and that the sarcoplasmic reticulum is a major storage site of calcium in both smooth and striated muscle.

G. Kirk (University of Chicago) demonstrated how the electron probe could be used in the analysis of single cells, by showing how the membrane transformations which distinguish red blood cells with a naturally high level of potassium from those with a

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naturally low level of potassium occur early in the differentiation of the erythroblastic stem cells. A. Lauchli (Technical High School, Hannover) using frozen-hydrated bulk root tissue, has shown that sodium is reabsorbed from the vessels in the proximal regions of the roots of certain plants.

Discussions on specimen preparation for microanalysis led to the conclusion that, with the possible exception of tightly bound elements, conventional wet chemical procedures were to be avoided and that low temperature techniques involving frozen-hydrated or frozen-dried sections, bulk samples or microdroplets of cell fluids were to be preferred. The only compromise might be to use freeze-substituted material or to encapsulate specimens in high molecular weight cryoprotectants before freezing. P. Echlin *et al.* (University of Cambridge) demonstrated that these polymers, which do not enter the cell, limit the size of intracellular ice crystals to nanometre dimensions, and do not seem to cause any significant loss of elements from cells and tissue, although some elemental redistribution may occur within the cell. The extent of elemental redistribution generally remains a problem, but there was agreement that valid elemental concentrations can be measured between different cell compartments, although it is probably premature to try and measure gradients and variations in concentration within a given compartment.

Several papers dealt with the quantification of analytical results. J. Russ (Prairie View, Illinois) described a new and relatively simple computer program for processing and displaying elemental spectra obtained from thin biological samples, and T. Appleton (University of Cambridge) discussed ways in which analytical information obtained from frozen-dried sections could be related to the fresh hydrated state. B. König (Batelle Institute, Frankfurt) considered the optimal conditions for high spatial resolution in the analysis of thin films and R. Bauer (University of Munich) gave details of a program for thin sections of biological tissues which separates elemental peaks and background radiation by means of artificially generated spectra. Using this technique together with internal albumin standards, R. Rick (University of Munich) found evidence for a syncytial sodium transport compartment in all epithelial layers of frozen-dried sections of frog skin epithelium.

There have been significant advances in the development of the laser microprobe mass analyser. R. Wechsung (Leybold-Heraeus, Cologne) gave details of a commercially available in-

strument with a spatial resolution of  $0.3\text{ }\mu\text{m}$ , detection limits of  $10^{-20}\text{ g}$  within the  $0.1\text{--}100\text{ p.p.m.}$  range and the capability of analysing both organic and inorganic material. F. Hillenkamp (University of Frankfurt) and R. Kaufmann (University of Düsseldorf) demonstrated how the instrument could be used to analyse trace elements and physiological cations and anions or for fingerprinting organic constituents.

Unfortunately, this high level of sophistication is still missing from the techniques of cathodoluminescence, and in the nuclear and proton probes. These techniques, together with ion probe analysis, still need further development particularly with respect to improving their spatial resolution and their biomedical applications. These are clearly the analytical techniques of the future and for once the biologists are ready to use them, having firmly established valid preparative methods based on low temperature techniques. □

## Economy and chemistry of phosphorus

from P. B. Tinker

A symposium, entitled The Economy and Chemistry of Phosphorus was held at the Ciba Foundation on 12–15 September, 1977, and chaired by Professor R. J. P. Williams. The Proceedings will be published by the Ciba Foundation.

PHOSPHORUS occupies a special place amongst the elements which are essential to life, because of the variety and complexity of the processes in which it takes part. The wide-ranging symposium was therefore of the broadest interest.

The first and most basic topic was supply, since there have been suggestions that phosphorus is a mineral resource which may be near exhaustion. Several speakers stressed the underlying problem of the explosion of population and economic expectations. The present facts are not in dispute: current production is some 15 million tons each year, rising on a long term trend of 6–7% per year, from minimum demonstrated reserves of some 6,000 million tons, which suggests exhaustion in 50 years. However, real reserves must be far larger, and J. W. Brinck (International Resources Consultants,

Alkmaar), used a modelling approach to infer that there could be anything from 40,000 to 500,000 million tons in all. Even this largest value would be exhausted in 120 years if the trend continues. As with all futurology, the numbers are so inexact that one can do little except show that rising trends cannot be sustained for ever. The real question is the mechanism by which these rising trends are stopped. As Brinck remarked 'Doomsday is always 70 years ahead,' and the best estimate seems to be that we have enough phosphorus in mineable reserves to take us beyond the limit of useful prophesy, especially since recent price increases have sharply reduced consumption. Possible technical developments in mining technology, the use of sea-floor phosphatic nodules, greater efficiency in the use of fertilisers and phosphorus recycling make prediction especially difficult, and it was suggested that our problems are more likely to be the familiar ones of ill-directed investment or the exercise of monopoly power. The analogy with oil springs easily to mind, but it is not really a good one: oil has several substitutes, although its withdrawal would be catastrophic at once, whereas phosphorus could be cut off without serious immediate harm, although it has no substitute at all.

By far the greatest part of mined phosphate is used for fertilizers, and G. H. McClellan (International Fertilizer Development Center), described the technology of their production, stressing how this affected the possibility of using low-grade ores.

The phosphorus cycle had a good run, with E. J. Griffith (Monsanto) discussing it on a global scale, J. C. Bowman (University of Reading) giving very detailed data for Britain, and A. F. Harrison (Natural Environment Research Council) dealing with discrete ecosystems. Natural ecosystems of forest and grassland have most of their phosphorus stored in the soil, with very small outputs and inputs, whereas the cycle in agriculture is much more 'open', with large inputs (as fertilizer) and outputs (as harvested crop). However, it is incomplete, in the sense that the inputs exceed outputs by a factor of between 2 and 10, due to adsorption of phosphate in the soil. It became quite clear during the meeting that this is the central problem in the world phosphorus economy, which consists largely of digging out phosphorus at one place and storing in the soil at another. The magnitude, value and use of this phosphorus store is not wholly understood, since the physical chemistry of phosphate in soil is extremely complex, but it is crucially important.

R. J. P. Williams (University of

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Oxford) introduced the consideration of the detailed role of phosphorus in biology, noting the vital functions it performs in the transfer and storage of information (as nucleic acid) and energy (as ATP) and in compartmentation (as phospholipids). B. C. Loughman (University of Oxford), followed with a detailed description of the uptake and metabolism of phosphorus by plants, the most notable point being the enormous ability of organisms to concentrate phosphate from extremely dilute solutions. This is important in the utilisation of 'soil store' phosphate, and crops can take this up, although it may not be able to support maximum yield. There is some possibility of breeding crops which are more efficient at absorbing low-solubility soil phosphate, and microbiological systems such as mycorrhizas are often able to assist phosphate uptake.

The importance of this uptake ability of organisms was illustrated by the very interesting and occasionally contentious section dealing with eutrophication of surface waters by phosphorus wastes—largely from detergents in sewage. The discussion centred on the Great Lakes, and G. R. Alexander (US Environmental Protection Agency) discussed the problem in general and political terms. There is agreement that phosphorus is the limiting element in all five Great Lakes, and removal of phosphorus from sewage inflows has proved unreliable. He saw little chance of stopping any of the other sources of supply, such as topsoil erosion, and a ban on detergent phosphates in sensitive areas would be the easiest method of improving the position. Members of the relevant industry naturally did not entirely agree. A much more relaxed view was taken by R. W. Collingwood (Water Research Centre, Medmenham Laboratory), who felt there was no justification for the high cost of removing phosphate from sewage in Britain. Our disposal problems are very much easier than those in America, and he emphasised that each case should be treated on its merits, rather than imposing blanket bans. The subject is extremely complicated, including political judgement of what 'eutrophication' is acceptable, and the great difficulty of predicting exactly what may happen in the sometimes complex ecosystems of lakes. On the whole, the real damage to the community resulting from either algal growth in rivers or from the withdrawal of polyphosphates in detergents seemed rather small in relation to the often passionate interest aroused by it. The very useful point was made that 'eutrophication' is really a state of higher biological productivity, which may be positively desirable, and that we should not

simply assume that the less phosphate the better, without thought.

The chemistry of phosphorus underlies all the other topics, though it was difficult to deal with it in depth in such a broad-based meeting. A. J. Kirby (University of Cambridge) discussed the fundamental facts of the transfer of the phosphate group, which is the essence of its biological importance. This deals with the P-O bond, and contrasted with the paper of T. D. Inch (Microbiological Research Establishment, Porton Down), who discussed the interesting compounds with P-C bonds. The phosphonates are important in the nerve gases and pesticides based on these, and they do occur in both plants and animals, though in very small amounts. Some of their substitution reactions are interesting in giving a retention of steric orientation.

R. J. P. Williams finally wound up the Conference, with a look back at the problems of unwanted phosphorus storage, and the desirable and undesirable growth resulting from its presence. The meeting produced no radically new ideas, but it formed an excellent synthesis of the state of knowledge at present. The wide spectrum of disciplines and interests made the discussion always interesting, sometimes fascinating, and showed up very clearly the different mental approaches, for example between the pure chemists and the 'field' scientists. □

## Photosynthesis at Reading

from J. Barber and B. Halliwell

The Fourth International Congress on Photosynthesis was held at Reading on 4-9 September, 1977. The Congress dealt with all aspects of research into photosynthesis under two broad categories; the light reactions, and carbon metabolism (including the physiological and applied aspects of the subject).

A SERIES of symposia brought together biophysical and biochemical work aimed at understanding how energy capture, transfer and trapping occurs in photosynthetic organisms. This area is highly active at the moment due chiefly to the development of new techniques and to the increasing use of

sub-chloroplast particles and purified reaction centres from photosynthetic bacteria. For example, G. Searle (Imperial College, London) reported how time-resolved transfer sequences between different pigments can now be studied in photosynthetic tissue using picosecond fluorimetry. P. Thornber (University of California, Los Angeles) emphasised that picosecond absorption studies are also providing valuable information on primary charge separation in bacterial reaction centres, and in particular referred to recent work with *Rhodospseudomonas viridis*. As yet, purified reaction centres from  $O_2$ -evolving organisms have still not been isolated and direct comparisons between these systems and the photosynthetic bacterial system can only be speculative.

Although the 'Z-scheme' for electron flow in  $O_2$ -evolving systems seems to be the best working model, there is still confusion about the role of cytochrome  $b_{559}$ , cytochrome  $f$  and cytochrome  $b_6$ . The function of these components must be understood before it can confidently be said that a full description of electron transport in photosynthesis is known. Moreover there may be other electron transfer components which have not been identified. The existence of a new component was suggested at the congress by R. Malkin (University of California, Berkeley). He reported that a 'Rieske' iron-sulphur centre exists in chloroplasts and from several lines of evidence suggested that it functions as an electron carrier between the plastoquinone pool and plastocyanin. Plastocyanin now seems to be fully accepted as a major electron carrier in chloroplasts and at the meeting the results of high resolution X-ray analysis of its structure was reported for the first time by H. C. Freeman (University of Sydney). Knowledge of the mechanism by which molecular oxygen is generated from water by light energy is progressing steadily. T. Wydrzynski (University of Illinois) presented some new results obtained with colleagues based on NMR studies. Their results suggest that the 'S-state enzyme' of the 'Kok-clock-model' contains four manganese atoms and is advanced by  $Mn^{2+}$  to  $Mn^{3+}$  changes. Whether their model is correct or not is open, but efforts to gain a chemical understanding of the reactions involved are welcome and moreover their model fits with studies of flash-induced  $H^+$  release presented by others at the meeting.

### Photophosphorylation

The symposium concentrating on photophosphorylation clearly demonstrated that in its broad terms, Mitchell's chemiosmotic scheme is ac-

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cepted by most workers. A few papers were presented which disagreed with some of its finer points and the exact values of the H/e or P/e ratios are still not known. Over the years H. Witt (Berlin) and his colleagues have presented strong experimental support for Mitchell's concepts and again at this meeting, Glaber from Witt's laboratory reported experiments in which ATP synthesis had been induced by creating artificial electrical gradients across chloroplast membranes. A requirement of the chemiosmotic scheme is that there is a membrane-located ATP synthetase or coupling factor able to do work when translocating protons down their electrochemical potential gradient. The coupling factor consists of several subunits and N. Nelson (Haifa, Israel) reported some unique experiments. He seems to have isolated the 'H<sup>+</sup>-ionophore' component of the complex and has tested its ability to increase H<sup>+</sup> conductance by incorporating it into artificial membrane systems.

### Carbon metabolism

The sessions on carbon metabolism dealt largely with the regulation of CO<sub>2</sub> fixation in C<sub>3</sub> plants and with photorespiration. No great advances in our knowledge of C<sub>4</sub> metabolism were reported, but it is now generally accepted that the Calvin cycle operates only in bundle-sheath cells. CO<sub>2</sub> is fixed in the mesophyll, transferred to the bundle-sheath as malate or aspartate and released again to be refixed by the Calvin cycle.

Regulation of the Calvin cycle is achieved by several mechanisms, one of which is a light-dependent increase in the activity of certain enzymes. Illumination generates dithiol groups within the chloroplast, which then activate the enzymes. L. Anderson (University of Illinois, Chicago) and B. Buchanan (University of California, Berkeley) gave evidence that both membrane-bound dithiol groups and the dithiol protein thioredoxin seem to

be involved. A tentative mechanism may be proposed (Fig. 1).

As. H. Heldt (University of Munich) reported, when chloroplasts are illuminated, the Mg<sup>2+</sup> concentration of the stroma increases by 1–4 mM and its pH from 7.0 to 8.0 as H<sup>+</sup> enters the thylakoids and Mg<sup>2+</sup> moves out. These changes regulate several enzymes of the Calvin cycle, especially fructose and sedoheptulose diphosphatases. Both enzymes work best at high pH and Mg<sup>2+</sup> concentration. Ribulose diphosphate carboxylase requires preincubation with Mg<sup>2+</sup> and CO<sub>2</sub> to obtain maximum activity *in vitro*; it is not clear if the enzyme is always fully activated *in vivo*. According to D. Walker (University of Sheffield) the kinetics of the activated enzyme can now account for observed rates of CO<sub>2</sub> fixation. J. Preiss (University of California, Davis) suggested that starch synthesis is regulated at the level of ADP-glucose pyrophosphorylase, which is activated at high phosphoglycerate/inorganic phosphate ratios.

Illuminated chloroplasts generate H<sub>2</sub>O<sub>2</sub> but contain no catalase activity. Ascorbate peroxidase together with glutathione reductase may remove H<sub>2</sub>O<sub>2</sub> *in vivo*, an argument put forward by D. Groden (Bayreuth) and by B. Halliwell (King's College, London).

Photorespiration is caused by the formation of glycollic acid and its subsequent oxidative decarboxylation. G. Lorimer (Munich) reported experiments with <sup>18</sup>O<sub>2</sub> which show that most, if not all, of the glycollate is produced by the hydrolysis of phosphoglycollate generated by the oxygenase activity of ribulose diphosphate carboxylase. U. Heber (Dusseldorf) also carried out other experiments which supported this conclusion. The glycollate so produced is oxidised to glyoxylate and transaminated to glycine in peroxisomes. The glycine is converted into CO<sub>2</sub>, NH<sub>3</sub> and serine in mitochondria. As A. Moore (King's College, London) explained glycine oxidation by leaf mito-



## A hundred years ago

THE chief signal officer of the U.S. army has been urging that physical observations of the sun be made, as of sun-spots, faculae, protuberances &c., in reference to their supposed influences upon terrestrial meteorology, and has offered to publish the results monthly, or such of them as may be considered desirable by the observer, in the *Monthly Weather Review*. The United States Naval Observatory at Washington has already accepted this proposition, and it is considered very desirable that some other observatories in the east and at least one on the western coast, cooperate in this undertaking.

From *Nature* 17, 8 November, 39; 1877.

chondria has a P/O ratio of 3; the NH<sub>3</sub> formed is probably a substrate for glutamine synthetase. Several groups of workers have shown that the amount of carbon flowing through glycine and serine in illuminated leaves is more than sufficient for glycine decarboxylation to account for observed rates of photorespiration (D. Canvin, Queen's University, Ontario; C. Whittingham, Rothamsted). However, Canvin also reported that the <sup>14</sup>C-labelling kinetics of glycine and serine are extremely complicated and inconsistent with a single origin for these amino acids, so it cannot be clearly stated how much photorespiratory CO<sub>2</sub> arises by glycine decarboxylation.

Refixation of CO<sub>2</sub> released by photorespiration consumes considerable energy within the leaf. G. Krause (Dusseldorf) thought that photorespiration may help to use up 'excess' light energy and protect the chloroplast from damage. As. E. Elstner (Munich) explained, the electron acceptor complex of photosystem I can reduce O<sub>2</sub> to O<sub>2</sub><sup>-</sup>, the toxic free-radical superoxide (Elstner, Asada). In the absence of CO<sub>2</sub> NADPH/NADP<sup>+</sup> ratios in the chloroplast will be high and so electrons should be shunted more rapidly to O<sub>2</sub>. Photorespiration, by making CO<sub>2</sub> continuously available for refixation, might help to decrease O<sub>2</sub><sup>-</sup> formation to a level that can be dealt with by the chloroplast's protective mechanisms. □

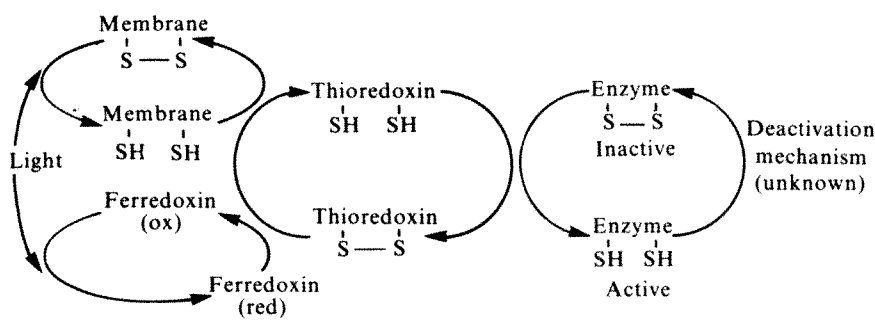


Fig. 1 Tentative mechanism for activation of Calvin cycle enzymes by dithiol groups generated by illumination of chloroplasts.



# Book Review Supplement

THIS year marks the centennial of Edison's phonograph; next year is the centennial of the Edison Electric Light Company (which General Electric claim as their birthdate); and 1979 will mark the hundredth anniversary of Edison's incandescent bulb. It would be possible to go on to suggest plausible Edison celebrations for each year well into the twenty-first century, thus giving strength to the claim that here we have the most prolific inventor of all time. Furthermore, many of his discoveries were fundamental contributions to devices which now dominate our lives, thus giving firm support to Mr Clark's subtitle.

The perspective of a hundred years should make it possible for the author, as promised on the jacket of the book, in writing about Edison "to present him clearly against the background of his times and to assess fairly what his achievements really were.". There are occasions in the book where a gesture is made in this direction—as on p14 where Edison's characteristics are equated with those of his age, or in the few instances of reference to the financial manipulations of his robber-baron contemporaries. But none of this is pursued. There are no fresh insights into the relationships between Edison and his family, or Edison and his workmen, or Edison and people like Jay Gould. There is the suggestion that after his first wife died his re-marriage to someone quite different significantly affected his attitude towards his work (p146). But even this is not amplified, and references to his work habits in later years seem to indicate a single-minded dedication virtually unchanged from the earlier period.

What we have, therefore, is a pleasantly readable biography, conventional in form, which offers no special commentary beyond what is available in other works. To me, it is not as satisfactory as the biography by Matthew Josephson, published almost twenty years ago (*Edison*, McGraw-Hill, 1959). This is partly due to the fact that Josephson allowed himself twice as many pages as Clark, partly due to the research he did on the Edison papers, and partly due to his familiarity with that period of American economic history. But Clark's biography shares with Josephson's a major failing in not describing in any significant detail the inventions that lay

at the heart of Edison's life. It is therefore difficult for the reader to assess the nature of the challenges facing Edison, or the ingenuity with which he solved them. There are a number of other more specific failings as well, and it seems worthwhile to mention a few of them here.

There is, for instance, the marvellous story of how Edison received a telegram from London in December, 1878, urging him to develop a telephone receiver that would circumvent the

additional thought and experimentation, and called for some assistance, there is no reason to believe he would turn the entire laboratory over to the project. Furthermore, during January, in the middle of this three-month period, Edison produced his first high resistance lamp.

Clark's account of the development of Edison's carbon-resistance telephone transmitter is not so much in error as it is misleading. Although noting (p58) that the patent application was made in 1877 and not granted until 1892, he dismisses the delay by stating merely that "it is symptomatic of the controversial situation." This was a very important invention (as Clark recognises), and it would seem reasonable to include some discussion of the controversy and of the parallel work of Emile Berliner, Francis Blake, and others.

Nicola Tesla, that most peculiar electrical genius, is given credit (p159) for developing the transformer and motor that made alternating current transmission practical. Although there is a considerable amount of truth to the statement, it suffers greatly from oversimplification, and a few qualifying words would seem in order. This is especially relevant because for the period in question—the late 1880s—the important work (on the transformer) was carried out by William Stanley for Westinghouse, with some debts owed to earlier activities in Hungary and Britain. On the same page, Clark relates that in 1912 Edison and Tesla were offered jointly the Nobel Prize in Physics, that Tesla spurned it because of his ill feelings towards Edison, and that it therefore went to someone else. This is a story that has several versions; the one which is Clark's source even has the date wrong. Whether the Nobel committee contemplated giving the prize to them is unknown, but in November 1915 the *New York Times* printed an account that this was the case. Others followed, and there were of course interviews with the two principals. No known statements by Tesla indicated any reluctance to accept the award; when asked why Edison might be honoured, he responded that Edison was worthy of a dozen Nobel Prizes.

The whole matter of the AC-DC controversy is given some prominence by Clark—and rightfully so—as marking a change in Edison's way of

## Ushering in the electronic age

Bernard Finn

*Edison, The Man Who Made the Future.* By Ronald W. Clark. Pp.256. (Putnam: New York; Macdonald and Jane's: London, 1977.) \$12.95; £6.95.

Bell patent. (Unmentioned is the fact that in Britain Bell's patent protection extended only to the particular devices invented, whereas in America he was allowed credit for the principle of voice communication by a fluctuating electric current.) In a *tour de force* that was so typical for him Edison did what was asked. He shipped off a working model within three months, thus allowing his supporters in England to arrive at a profitable understanding with the opposition. Clark tells this story, and tells it well. After noting, however, that this was a time when intensive efforts were being expended on the incandescent lamp, he tries to make a point about Edison's methods of operation, stating that he "acted promptly and ruthlessly. He withdrew all his men from work on the electric light and put them on telephony." (p59). But this was surely not the case. The new receiver (which depended on the varying amount of friction that was produced when a varying current passed between a rotating drum and a stylus pressed on it) was directly related to an earlier telegraphic instrument he had invented; and although Edison undoubtedly expended a good deal of





Photo: Edison National Historic Site, Orange, New Jersey

Edison with two of the lamps produced to illustrate the Edison effect, later to become the basis of the electronics industry

thinking. This was during the period, already mentioned, when he remarried; an analysis of how various pressures then came to bear on Edison, and how he reacted to them, would make extremely interesting reading. Unfortunately, there is no real attempt to do this. Even the account of the succession of events is marred by errors of omission and commission. For instance, unmentioned by Clark, there were some very good reasons supporting Edison's side of the argument: storage batteries could be used in conjunction with direct current generators to even out the load cycle, and no motors had (yet) been invented to work on alternating current. The eventual capitulation—a merger with Thomson-Houston—is presented (p179) as if Edison were completely unaware of what was transpiring. Although it is true that Edison was against the merger and was largely kept in the dark, he had been aware for some time of the state of negotiations. His strong reaction, cited by Clark, was to the revelation of the final details, which merged everything together and stripped his name from the resulting firm title of General Electric.

There is no reason to believe (p65) that weakness in theoretical training

caused our hero to miss an opportunity to exploit the Edison effect. This was his discovery in 1883 that negatively charged particles were given off by his lamp filaments, and that they could be attracted to a positively charged extra electrode. He carried out a number of experiments and then applied for a patent where the effect was used in a sensitive current detector—the only use he could see for it. The experiments were known to physicists and electricians throughout the world, and none had a better suggestion. John Ambrose Fleming experimented with Edison-effect bulbs over the next few years before also abandoning them. It was two decades after the discovery (1904) before Fleming applied the Edison effect to act as a moderately effective detector of radio waves, and another decade before such a device (with a third electrode, added by Lee de Forest in 1906) was successfully used in an amplifier, thus ushering in the electronic age.

There are numerous other examples of this same type, and if one were to draw a moral from them I suppose it would be that scientific and technical biographies are very difficult to write. Further evidence for such a statement is the fact that very few have been written. If anyone should know this, it should be Mr Clark, who has specialised to a degree in this sort of writing. In his recent biography of Einstein he minimised the weakness of his own scientific background by doing a considerable amount of legwork, seeking out unused written material and interviewing people, to provide the makings of a dimension to the person he was describing that had not been available before. This could have been done with Edison. If Clark had used his strengths as a biographer to probe Edison's character and his dealings with other people, then the results might have been of great interest.

The fact of the matter is that the corpus of Edison material—most of it preserved at the historic site in West Orange, New Jersey—is enormous. The task of dealing with it intelligently in its present form, in spite of the amount of organising effort that has already gone into it, is far beyond the capacities of any one person. A fitting centennial tribute to Edison would be the inauguration of a project to develop these papers in a more useable fashion and to make them more widely available. There is hope that this will happen. If it does, the next biographer will be able to reveal to us a great deal more about the man who made the future. □

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# Evolving a good bedside manner

Don Brothwell

*Origins: What New Discoveries Reveal About the Emergence of Our Species and Its Possible Future.* By Richard E. Leakey and Roger Lewin. Pp. 264. (Macdonald and Jane's: London; Dutton: New York, 1977.) £8.95; \$17.95.

THERE is perhaps some wisdom in the idea that before books are written, the authors should discuss the contents with potential reviewers. In the case of a detailed monograph or lavishly illustrated coffee-table book, one knows pretty well where one stands in terms of how to review it. In the first case one evaluates it particularly in relation to the limited number of specialist academics or students who will consider it; at the other extreme, the acid test is whether it will hold its own against Scotch, conversation or the television. But the hybrid is a problem, and that is really what *Origins* claims to be. The publishers tell us that it is "handsomely illustrated and fascinating to read" as well as being "filled with controversial ideas and fresh insights". Oh dear, a difficult one, pitched at the general reader but putting out new ideas which are presumably for professional eyes as well. The only answer is of course to try and play the same game. Is it pleasant reading for any of us interested in the evolution of our species (and most of us pretend to be, even though the state of 'advanced' societies suggests that few really are)? Secondly, does it really contribute something new to this well-trodden field, beyond a few new pictures and a few extra basic facts (easy enough to produce in human palaeontology)?

But to begin at the beginning, the sequence of chapters follows a pattern which is fairly standard now in books concerned with the evolution of man. We see primate evolution scaled against the few thousand million years of emergent life generally, a fact which should really put us firmly in our place and give us a strong perspective for viewing the biosocial absurdities of our own species; but it never seems to. The history of evolutionary controversy and theory is scanned briefly, reminding one that bias is all too easy and that there is a need for a detailed study of the history of palaeoanthropology, with

not only Darwin, Huxley, Owen and Dart, but also Elliot Smith, Ruffer and many others who were not only concerned with evolutionary theory but were also working on such questions as neurological change and ancient disease—exotic perhaps to some but just as relevant to the proper appreciation of the biology of past hominid populations.

Viewing the primates as a whole over their seventy million years or so of evolution is also an exercise of use to the hominid palaeontologist, setting his own distinctiveness and rates of change against that of other species within the Order. There are clearly explosions of adaptive success; first the prosimians, then monkeys, followed by a rather modest hominoid (ape-plus-human) differentiation, and finally the population explosion of modern man himself. In the interests of easy reading, considerable opportunities are missed for showing the questionable nature of much of the work and findings in this field, and the great research challenge which remains. But this is always the problem in popularising science, and although perhaps a lot of the fun of research is in the uncertainties, challenges and points of disagreement with colleagues, perhaps this does not lead to good reading at a general level? I confess that I think it does, but admittedly detailed and truthful revelations about some controversial issues would have to be very skillfully and carefully written indeed in order to avoid multiple libel actions.

Although much of the book seems to highlight Africa as a focal area of palaeoanthropology, the chapter on hominid beginnings rightly points out that most apparent ramapithecine material, a possible early hominid of about ten million years ago, is found outside Africa. This seems to be a rather neglected yet very significant fact, and if this group is indeed part of a variable stock from which the later hominids were derived, there is no reason why, in the following three or four million years, this group could not have given rise to advanced hominids in Europe or Asia, rather than Africa alone.

The next chapter, "The Cradle of Mankind", is concerned with a further morphological phase seen in human evolution—the australopithecine and *Homo habilis* groups of Africa. But how can one be sure, in this early stage of field-working world Plio-Pleistocene deposits, that many sediments out of Africa will not eventually produce these or related hominid varieties? And even if relevant fossils do not appear soon in other continents even though many more do in Africa, what does this

mean? From the evidence of skeletal material excavated in Britain, one would have to conclude that the Scots and Welsh hardly existed and only southern Britain was generally populated over the past few thousand years—a fact really correlated with soil acidity rather than historical demographic truth.

As expected, the fossils grouped under the name of *Homo habilis*, and especially the 1470 skull from Lake Turkana, are discussed in some detail. The fact that a number of deposits have now produced evidence of this "primitive *Homo* of nearly four million years old" is certainly interesting but whether "Just a few years ago no-one in their right mind would have believed this possible" seems debatable. I for one considered it possible and am not noticeably of unsound mind.

If one views human evolutionary studies from the turn of the century, it can be seen how the timescale has been greatly extended. In this respect at least, we tend to be rather conservative, although there seems to be an easier acceptance of much larger dates now, and indeed there is perhaps even a growing feeling that we must give as much time as possible for the mosaic of changes we know to have taken place in the limbs and pelvis, skull and brain. For, whereas Sir Arthur Keith, writing fifty years ago, could consider morphological evolution as if it were a sequence of easy growth changes, we must now see it as a complex product of multiple gene pool changes linked with environmental demands and social development.

Early tools are rightly given space in various parts of the book, and the comments on the early evidence is of course especially interesting. The great problem is to know what some of these roughly made objects really mean in terms of cultural development in the community. We can watch nest construction in birds or object manipulation in chimpanzees until we are blue in the face and still be quite unable to usefully interpret the significance of a remnant stone technology in another species where the brain is known only from an endocast. What is particularly tantalising is that one suspects that tools such as the poorly manufactured quartz objects from the Omo River Valley are telling us more than we can yet cope with in terms of early hominid perception and language development, the education of the early hominid child into the practicalities of hunter-gathering, and even the emergence of more advanced forms of group co-operation.

With the discovery of the fairly complete *Homo erectus* cranium from



East Turkana, doubts as to the considerable antiquity of this species are rather cleared away. (Claims for an earlier date from Java have been viewed with some uncertainty.) Whether Turkana Man was "very similar" to Peking Man is rather debatable, but that does not affect the importance of this find, and I would have liked to read more comments on this and related African specimens. For that matter, the authors could have usefully drawn in more of the *Homo erectus* and later Middle Pleistocene specimens which have been discovered in the past decade or so; and indeed I felt that the book sadly underplayed some discoveries in non-African areas of the world. I confess to being reluctant to accept their scheme for the emergence of *Homo erectus* in East Africa and then derive European and South-East Asian representatives from this stock. Perhaps this is only the result of a suspicious mind refusing to believe that the early hominids of between two and five million years ago were so restricted to Africa.

More than one chapter is concerned with this level of evolution and in the

one on "Intelligence, Language and the Human Mind" the authors give an opinion that "the vocal apparatus of *Homo erectus* would have enabled him to speak in a slow and rather 'clumsy' fashion". This brief but understandable comment is perhaps the essence of popular writing and perhaps does no harm, even though it is very misleading as regards the certainty of this fact and the lack of good evidence to substantiate it.

After considering the more recent phases of human evolution, including such aspects as population increase, agriculture, aggression and rituals, the authors are still able to conclude optimistically. And perhaps it is all to the good if palaeoanthropologists do cultivate a good bedside manner, cheering us on instead of placing us in the shadow of the dinosaurs. Unfortunately, I suspect that our mission may eventually be that of Job's comforter.

Leakey and Lewin have produced a nice book for the general reader, not one for the student or professional. □

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## Fanatical system-builder

A. J. Cain

*The Spirit of System: Lamarck and Evolutionary Biology.* By Richard W. Burkhardt, Jr. Pp. 285. (Harvard University: Cambridge, Massachusetts and London, 1977.) £11.55.

It is a great relief to have at last a good book on Lamarck in English, neither partisan through patriotism, nor distorted by dislike of Darwin. Burkhardt gives us an excellent introduction to Lamarck, his contemporaries and predecessors, and to the scientific bodies and institutions in revolutionary Paris. Anyone working on Lamarck is necessarily limited by the almost total lack of biographical and personal material, but Burkhardt has gone carefully through the published writings and the manuscripts of Lamarck at the Musée d'Histoire naturelle, and those of Cuvier there and at the Institut. He has read widely in the literature of the period, and his sketches of the ideas and opinions of Daubenton, Faujas de Saint-Fond, Buffon, de Jussieu, Bruguière, and Lacépède, for example, are essential for understanding the variety of opinions and the wonderful richness of the biological, physical and chemical work in Paris in Lamarck's lifetime.

On Lamarck himself Burkhardt gives a useful sketch of his life and career, and the best analysis both of his evolutionary ideas and the way in which he altered his opinions, or at any rate some of them. The title of the book refers to that passion for system building (esprit de système) which had already been recognised as a major obstacle to scientific progress—Lamarck would not have liked its application to him at all, but it is justly used. He was a fanatical system-builder, far too hasty, far too impatient to test the validity of the 'facts' he built on, far too tenacious of dubiously correct ideas, and incredibly opposed to the best physical science of his day. He was, in short, a first-class

crank. One is reminded of Alfred Russel Wallace on anti-vaccination, life on Mars, spiritualism, land nationalisation, and the flat-earth people. Cranks are usually disastrously wrong, but when they are right, because of their fanaticism they are righter than most others—in some respects Wallace's *Darwinism*, which rather surprised Darwin, was better than the *Origin of Species*; and he was of course a great biogeographer. Lamarck also has plenty of claim to biological credit, for his taxonomic work; but his system of thought on the nature of life and on evolution reposed on such hopeless physical, chemical and geological foundations, and was presented with such disregard for scientific sobriety, that it is no wonder he was rejected by his contemporaries. One cannot but have sympathy with him for his unfortunate life, his blindness, his miserable last years; nevertheless, he was a crank. Burkhardt approaches him with too much deference to say anything of the sort, but he does indicate at the end that Lamarck cannot be considered a man ahead of his time.

Lamarck began his scientific work as a botanist, adept at constructing keys and fully aware of their artificial nature, believing in the immutability of species, and convinced that within the plant and the animal kingdoms separately there is a serial order which is the natural order. He knew well that both plants and animals vary considerably, often in relation to different climatic or cultural circumstances. He knew, as did everyone, that there was a balance in nature, such that all species continue, acting as checks on each other by predation; none, he believed, were lost. Two of these ideas, somewhat battered, he clung to to the end of his life—that within animals or plants there was a single natural serial order, or perhaps two; and that except for some large animals perhaps extinguished by man and the hordes of infusorians which could be easily destroyed by variations in external conditions, no extinction had taken place. It was this last conviction that, as Burkhardt shows very clearly, drove him to the idea of mutability of species—if it could not be that large numbers of species had become extinct, then they must have transformed into other species.

Right from the beginning, Lamarck had been thinking about the nature of plants, their constitution and physiology. He was especially impressed with the influence of climate, soils, and other environmental factors on them, and he started to think out a general theory of living and non-living things. It would be fascinating to look at his Jesuit schooling, which might



Cuvier



in part explain some of his ideas in physics and chemistry. He clung all his life to a thoroughly idiosyncratic four-element theory (of earth, air, fire and water) combined with those subtle and invisible fluids, magnetism, electricity, and so on, which were the common property of almost all scientists in the eighteenth century. At first he thought of the origin of the world, and the nature of motion and of life as wholly mysterious, but before he finished he could explain the powers of life entirely in terms of his own physics and chemistry. He also believed that matter without the action of life had no tendency to form compounds. Complex matter left to itself would continue to disintegrate, and he produced a degenerative series beginning with excretions, secretions and corpses, ending with the purest of all solid matter, rock crystal. Burkhardt considers the well-known antagonism between Cuvier and Lamarck to be a consequence perhaps of the former's religious views but also because he had a philosophy of stability antagonistic to Lamarck's ideas of change. It is as likely that Cuvier was a better scientist than Lamarck and that his ideas were better founded in the practical science of the period.

Lamarck's observations in geology had convinced him that the Earth had undergone very long slow gradual changes; he was an ultra-uniformitarian and therefore could think of no mechanism for large-scale extinction. He was also a good conchologist, and Burkhardt brings out well the importance of this subject for problems of extinction at the time—Faujas de Saint-Fond, struggling with it, was so eager for Lamarck's conchological system that he worked from the proofs of it. On the one hand, many species seemed to be extinct but to be related fairly closely to present ones; on the other, forms thought to be extinct occasionally turned up alive (a trigoniid claim in Australian waters, for example); and vast areas of the world were still unexplored. It was a difficult question, more so for Lamarck because large numbers of intermediate forms, which should have existed on his theory, had not come to light. Why he should have clung so tightly to non-extinction is not explained. I suspect it was because, as an ardent Deist he could not contemplate serious imperfection in the world. His over-riding faith, that everything happens by law, surely springs from this attitude and from the gigantic success of Newton. Probably, his belief that the time was ripe for a synthesis of all biology from the facts then available came from an unfortunate hint of Newton's that also influenced Erasmus Darwin deeply.

Once convinced that transmutation of species must have occurred, Lamarck immediately found a dual mechanism for it, a purely physico-chemical system, whereby life, once started in simple specks of matter, would through the circulation of fluids cause expansion, then channels for the fluids, then further complexities of organisation. This, by itself, would produce a uniform series of living things beginning daily with spontaneously arising animalcules, and ending after an incredible period of time with Man, the most perfect of all. But each animal, or plant, has to live in particular ways, and their circumstances would act to modify them for their roles. This would explain neatly why there is, ac-



cording to Lamarck but not Cuvier, an overall progression in the great groups of animals or plants but much difficulty in making serial arrangements of species or genera within them. This mechanism of adaptation was again a commonplace of the time, the inheritance of acquired characters. Lamarck said explicitly that he prided himself not on this mechanism but on seeing its enormous implications, which no-one else had done.

Lamarck had no reputation in France until it was necessary to find a national rejoinder to Darwin. Much of what was then written was uncritical in the extreme. Since about 1950, many excellent papers have appeared in various languages, and one can now hope to look at the natural history of Lamarck himself. This book is a major contribution, but it suffers somewhat, like so much written recently by historians and philosophers, from a lack of biology. For example, Burkhardt says that Lamarck's theory of evolutionary change was a specially eighteenth-century one, seen also in

Adam Smith, Hume, or Rousseau, in which a natural process takes place, but it is affected by constraining circumstances. The implication is that he was a child of his time, which in general is certainly true. But nevertheless he and all other biologists knew that the embryo in the uterus develops and complicates enormously in a nearly constant environment; there must therefore be a power of development independent of environmental fluctuations. He knew also that plants and animals when domesticated might change surprisingly; there must therefore also be a power of the environment over the results of development. It is difficult to see why this is a specially eighteenth-century interpretation.

Equally, there are moments when one wonders whether the author appreciates what Lamarck is referring to. Burkhardt mentions Lamarck's pointing out that "the isochronous movements of the large, soft radiarians" is like the movement of the liquid in a heated thermoscope. What Lamarck is referring to is simply the pulsations (as they swim) of big jellyfish, which will be familiar to most readers. Burkhardt stresses the importance of conchology, but does not give us any insight into what it means to Lamarck in his daily work and thought, apart from the example of the trigoniid already mentioned. Too much history of biology written by historians and philosophers omits too much of the biology. A history of ideas is good in itself, but in science, as against philosophy, those ideas have a practical context within the subject matter of that science which cannot be ignored. We have been shown far too often how scientists are the creatures of their times (which is true) and far too seldom how they are also influenced by their subjects. One possible source for Lamarck's ideas of fluids circulating and 'forming' vessels for themselves might be found in the teratological and embryological knowledge of the period.

The book is exceptionally well written, with only a few infelicities of translation (brute matter is not a good translation of *matière brut*, and conscience in French is not conscience in English) or idiom (no-one did anything in 1797, only *as of* it, an unnecessary pseudolegalism) or checking (notes 88 and 89, chapter 5, and note 1, chapter 6 are incorrect references according to my copies of Lamarck). It will be an indispensable introduction to the subject, and the basis on which an examination of Lamarck's actual biological knowledge can be reared. □

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# Homo exquisitus

R. D. Meikle

*Ehret: Flower Painter Extraordinary.*  
By Gerta Calmann. Pp. 160. (Phaidon:  
Oxford, 1977.) £16.

THE history of Georg Dionysius Ehret (1708–1770) exemplifies the plight of many a botanical artist, dismissed (until recently) as a technician by the world of Art, and used, but scarcely appreciated, by botanists, to whom such elegant work seems to be of little more than peripheral importance.

Ehret was born in Heidelberg (or possibly Erfurt), the son of a gardener, with no advantages of birth save an observant eye and a natural talent for drawing and painting flowers. After a brief apprenticeship in horticulture, he turned to botanical illustration, serving a succession of patrons, who, it would seem, were rather more anxious to exploit his genius than to reward it. In many ways, his life resembles that of Mozart: plenty of appreciation, and no lack of hospitality, but probably always an acute shortage of cash. Like Mozart, his strivings for a measure of independence were labelled giddiness by his betters: "he is homo exquisitus in everything, he has only one fault, he is flighty".

His end was not a tragic one, perhaps because he had the good sense to head for England, where in time he became something of a celebrity, commending himself to Mrs Delany and the Duchess of Portland, and no doubt discovering that nothing is more acceptable to the English than an imperfect command of their language; for, even after thirty years' residence, he could barely make himself understood. In view of his reputed flightiness, even the daunting task of teaching the daughters of the nobility how to draw and paint flowers may have had its rewarding moments.

From his arrival in England, in 1736, until his death thirty-four years later, Ehret's life seems to have been tolerably pleasant, and was certainly very productive. It was not, however, the sort of life that was likely to contribute to his lasting fame. Much of his best work remained unpublished. Splendid paintings on paper and vellum may have given momentary gratification to the proud owners of stoves and exotica, but they were soon filed away and forgotten. The published engravings, even those in Linnaeus's *Hortus*

*Cliffortianus* and his own *Plantae et Papiliones Rariores* can scarcely be judged more than competent, whereas those in Trew's *Plantae Selectae* and *Hortus Nitidissimus* are sometimes mere travesties of the originals, almost as lifeless as the text which they accompany. Furthermore, the very works which Ehret illustrated ceased to be of more than scholarly and historic interest once the simple binomial system of plant nomenclature had been fully grasped.

Ehret was the contemporary (and avowedly the friend) of Linnaeus, but, in company with many of his patrons, he failed to appreciate the Linnean revolution in plant-naming, and continued to cling to "pre-Linnean" phrase-names long after the publication of the *Species Plantarum* (1753). It is for this reason that the writings of Miller, Aiton and even the ingenious Hill are still valued, whereas those of Trew and Weinmann were outmoded almost as soon as they left the press. Had it not been for the recent revival of interest in Ehret as an artist, there can be little doubt that his eclipse would have been total. In the unsympathetic world of taxonomy, he is, even now, little more than a penumbra.

Dr Gerta Calmann's monograph should dispel some of the shadows. But Ehret is a difficult person to write about. He was forever on the move, flitting, like one of his butterflies, from flower to flower and from patron to patron, without leaving any record of his activities save numerous signed and dated pictures and a brief autobiographical sketch specially designed to ease his entry into the Imperial German Academy of Naturalists. To make sense of such a complex and disjointed career, it is necessary to plan carefully and prune rigorously. Unfortunately,



Dr Calmann seems to have caught some of the flightiness from her subject. The text is widely informative, and often entertaining, but however much one may be amused by the antics of the Marcgrave of Baden-Durlach and his troop of female hussars, these, and similar digressions, do not help to concentrate one's mind on the essentials. Moreover, the author's frank admission that she is not a botanist does not excuse the astonishing string of errors on p48. Here, we are told that, "In a flash of insight Linnaeus realised that stamens and pistils were the sexual organs of plants", that his "artificial" system "was superseded finally by Darwin's theory of evolution", and that Linnaeus "created botanical Latin which simplified the identification of plants". Such *obiter dicta* do not necessarily impair the validity of the remaining text; but they undermine one's confidence.

The coloured illustrations, despite some, possibly unavoidable, reduction, are generally good, certainly very much better than many of the reproductions which appeared in Ehret's own lifetime. They have been selected to span almost the whole of his career; and although they do not demonstrate any remarkable development in skill or style, they do, fairly if rather unkindly, show the artist at his best and at his worst. His quality is certainly uneven, some of the plates, especially the Long-leaf Pine (pl.46) and the Christmas Rose (pl.95) being models of freshness and grace, whereas others, notably the Rose (pl.7) and more particularly the Poker-plant (pl.54) are so wooden and opaque that it is hard to believe they are by the same hand. The flattened, dive-bombing butterflies which appear in so many of his pictures are not, to my mind, an adornment; out of charity, one hopes they were forced on the artist to satisfy the catholic tastes of his naturalist patrons.

The monochromes are on the whole less satisfactory. Those which are allowed a full page are acceptable enough, though even here the "Characters of Flowers" (pl.75) with its amusing reminder of Ehret's faulty English, lacks much of the crisp quaintness of the original. The quarter- and half-page monochromes lose so much through reduction and absence of colour that one wonders if their replacement by a few additional colour plates would not have been wiser. The obscurity of one of these little illustrations (pl.16) may explain the misidentification of Thorow-wax (*Bupleurum rotundifolium*) as Yellow-wort (*Blackstonia perfoliata*). □

R. D. Meikle is on the staff of the Herbarium at the Royal Botanic Gardens, Kew, UK.



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## South African cheer

K. L. Manchester

*A History of Scientific Endeavour in South Africa.* (A collection of essays published on the occasion of the Centenary of the Royal Society of South Africa.) Edited by A. C. Brown. Pp. 516. (Royal Society of South Africa, c/o University of Cape Town, 1977.)

ON 22 June 1877 in Cape Town was founded the South African Philosophical Society, which on 25 January 1908 by grant of Royal Charter from Edward VII became the Royal Society of South Africa. *A History of Scientific Endeavour in South Africa* has been written to commemorate the centenary of the Society, which is the oldest body of its kind in the country—probably in the African continent as a whole. Edited by A. C. Brown, Professor of Marine Biology at the University of Cape Town, the book consists of a series of historical essays by some of South Africa's leading scientists, recording the efforts of workers in many fields who have broadened knowledge and understanding of the South African habitat.

The Foreword by the State President describes the book as a tribute to the many who have contributed much of the basic knowledge and research that has made possible the transformation of South Africa from the remote and little-known land of hunters, pastoralists and subsistence agriculturalists it was in the seventeenth century to the economically advanced nation that has emerged in the twentieth—one of the world's leading producers of many minerals and the leading industrial nation in Africa. This may all sound a little like advertiser's copy, but as one of the contributors to the volume concludes: "It is doubtful whether many other countries can show a similar growth of scientific institutions and of the solid body of original work carried out by them, if one takes into account the size of the population."

Of course, South Africa has in some ways had things made for it. The enormous mineral deposits have been provided by Nature, even if exploited by man. The clear skies have attracted the astronomers and the wealth of wildlife the biologists. Not only is the wildlife extremely rich and varied but many of the species are endemic, due to the relative long isolation of parts of South Africa by desert from the rest of the continent. It is interesting to read that even in the early 1800s there was a

considerable demand for South African wild animals in Europe, and their export, both dead and alive, was a profitable business. Gone are the days when the springbok trekked over the Karoo in vast herds to the delight of the hunter. Wildlife conservation is rather better arranged today, and the game reserves of South Africa must rank amongst the best managed in the world.

More academically, in *Australopithecus africanus*, South Africa has fulfilled Darwin's prophecy that Africa would prove to be the home of the 'missing' link, and lately evidence of the presence of the more advanced *Homo habilis* has come to hand. The Karoo Formation is also remarkable for its wealth of unique fossil reptiles and the east coast waters for the coelacanth.

Inevitably, many of the chapters read like a catalogue of names and events which for the average reader will prove difficult to assimilate. Nonetheless it is impossible not to perceive the steady growth through the years of the various scientific disciplines from the incredibly primitive to the sophistication of the present day. The early settlers suffered grievous losses of crops and stock due to unfamiliar pests and disease. Pains-taking veterinary and agricultural research has overcome many of these problems, vastly extending the extent of habitable country. Institutions such as Onderstepoort, built up by the redoubtable Theiler, have a worldwide reputation.

Proper medical education in South Africa only began in 1912. Yet today, the Medical Schools of Cape Town and the University of the Witwatersrand stand amongst the best. *Xenopus laevis*, the South African clawed toad, has made its mark in modern molecular biology; but to a wider public the discovery in Cape Town in 1933 that *Xenopus* could be used for a bioassay for human pregnancy has possibly been of greater significance.

The exploitation of mineral resources naturally has stimulated engineering and metallurgical research. Rockbursts have to be understood if they are to be avoided. Pursuit of the gold bearing reefs to ever increasing depths has given South Africa unique experience of the engineering problems of deep level mining and the health hazards involved. Mining is a great consumer of energy, but South Africa is blessed with 60% or more of all African coal resources; and neighbouring territories have considerable hydroelectrical potential. We may no longer dream of a Cape to Cairo rail link, but electrical reticulation of southern Africa is today of considerable logistic significance. Lack of oil is the Achilles heel of the South African economy. In the SASOL

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coal to oil process the country seeks self-sufficiency both for oil and petrochemicals.

A final chapter is devoted to the history of the Royal Society itself. It is amusing to find that in 1902 and 1905 the Society made a strong case for metrication of weights, measures and coinage in South Africa, an attempt that failed due to pressure exerted in the British Parliament. The Society was 60 years too early.

It is impossible to summarise in any meaningful way the mass of detailed material contained in this volume. To

anyone with an interest in South Africa or in the historical concept of how a country can develop scientifically the book is a must. The Royal Society of South Africa is to be congratulated in seeking to put together for the first time the fascinating story of the growth and discoveries of science in southern Africa. Into an atmosphere of political gloom and frustration it injects a note of human achievement and cheer. □

*K. L. Manchester is Professor of Biochemistry at the University of the Witwatersrand, Johannesburg, South Africa.*

## Historical revisionism

M. J. S. Rudwick

*The Making of Geology: Earth Science in Britain, 1660–1815.* By Roy Porter. Pp. 288 (Cambridge University: London, Cambridge and New York, 1977.) £8.95.

MOST Earth scientists are conscious of the great conceptual integration that plate tectonic theory has brought to their science in the past two decades. In retrospect, the immediately preceding period seems like a time of relative stagnation or, at best, of several disparate disciplines evolving in relative isolation.

This situation is not unlike an earlier period, to which historically conscious geologists often look back to find the origins of Earth science as a whole. In doing so, however, they generally adopt an historical interpretation that was itself a product of that period. The scientists who first gave 'geology' its institutional and cognitive identity in the early nineteenth century looked back to the eighteenth and late seventeenth centuries as a period of benighted obscurantism or fanciful speculation, relieved only by a few isolated

pioneers or forerunners of their own approach.

This is the interpretation that Dr Porter has set out to demythologise. His book is a conscious piece of historical revisionism, yet in no sense is it a facile exercise in debunking. His starting point is the striking discrepancy between the negative evaluation that nineteenth-century geologists gave to the work of their predecessors, and the actual content and scope of that work. The latter is reconstructed by historical analysis of an impressive array of published books and articles, unpublished field notebooks and correspondence, and other material, illustrated by often fascinating quotations. Dr Porter suggests, in my opinion persuasively, that early nineteenth-century geologists rejected or disowned the past history of their subject, because they felt they had successfully constructed a self-sustaining social enterprise with a new research programme of unlimited potentialities—significantly, the very word 'geology' only came into general use at that time. They contrasted this with the relatively undirected and even confused activities of their predecessors. Failing to see how these activities had been an indispensable precondition for their own work, they exaggerated the contrast into an almost total discontinuity.

*Making* is the key word in Dr Porter's title and in his interpretation.

Although the Earth was in an obvious sense always 'there' to be studied, the body of theoretical ideas and practical techniques that characterised the new science of geology was constructed out of the choices and decisions of individuals and groups in specific historical circumstances. To put it another way, the manifest heuristic success and cumulative character of the mature science of geology since the early nineteenth century is a historical phenomenon that calls for explanation, just as much as the 'failure' of earlier generations to create such a self-sustaining enterprise.

Dr Porter's approach is one in which the traditional dichotomy between 'internalist' and 'externalist' history of science is shown up in all its sterility. It is pointless to trace the growth of a community of like-minded 'geologists', or the reasons why that group developed when and how it did, without analysing the theories and techniques to which their likemindedness was directed; but the converse—the style of traditional 'internalist' history of science—is equally futile. Dr Porter succeeds admirably in blending context and content into a single integrated interpretation.

This book is not, and does not claim to be, a definitive or exhaustive description of all the research that was carried out during the period 1660–1815, even within Britain. It is a suggestive outline of a new way of looking at the early history of the earth sciences. The first appearance of a self-conscious science of geology is not seen as a product of an intrinsic progressiveness of knowledge or of the genius of a few pioneers—interestingly, the Scottish natural philosopher, James Hutton, still sometimes called 'the founder of modern geology', is shown to have been somewhat isolated from the mainstream development of the science. Instead, the emphasis is on the purposeful construction of an integrated discipline with clearly defined methods and—at first—quite narrowly limited cognitive aims; and this construction is seen as the work of individuals and groups working in specific circumstances that constrained the social forms in which their activity was embodied.

The geology that had thus been 'made' by the early nineteenth century is recognisably continuous with the earth science of today, even after its most recent 'revolution'. Dr Porter's account of this construction can be warmly recommended to all Earth scientists who are interested in the foundations of their science. □

*M. J. S. Rudwick is Professor of the History of Science at the Free University, Amsterdam, The Netherlands.*



Two views of Scottish geologist, James Hutton (1726–1797)



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# Philosophical conflict

Peter Winch

*Wittgenstein and Scientific Knowledge: A Sociological Perspective.* By Derek L. Phillips. Pp. 248 (Macmillan: London, 1977.) £10.

THE aim of Professor Phillips' book is to bring Ludwig Wittgenstein's philosophical work to the attention of sociologists, in the belief that it has something to contribute to issues which exercise them. It is concerned mainly with certain issues in the general philosophy of science which have recently come to the fore in the conflict between the hitherto dominant positivist and Popperian conceptions of science and the work of such writers as Thomas Kuhn and Paul Feyerabend. These issues touch the work of sociologists most directly in the area of 'sociology of knowledge', stemming from Karl Mannheim. As Phillips observes and brings out, many of Wittgenstein's ideas are very germane to such issues: particularly, the notes he wrote towards the end of his life published in *On Certainty*. The two issues in the philosophy of science and sociology of knowledge on which the book under review focusses most directly are: (1) relativism and the claim, arising especially out of Kuhn's work, that theories separated by a 'scientific revolution' are incommensurable; (2) where does authority lie in science: who, that is, decides what is respectable science and what isn't? Phillips has interesting things to say about both these topics, especially the first, though his treatments of them are not entirely consistent with each other. But I shall return to this point later.

The structure of the book is loose and not altogether perspicuous. There are chapters which are exclusively expository of some of Wittgenstein's main philosophical ideas; chapters in which some of these ideas are brought to bear directly on those issues in the sociology of knowledge in which Phillips is most interested; a chapter in which those issues are treated without explicit reference to the relevance of Wittgenstein's work to them. Overall, this gives an impression of disjointedness: the various issues discussed do indeed have important interconnections, but they are too often presented in indigestible chunks, and not enough work has been done to work them into a coherent whole. This is especially true of the

relationship between the first chapter on 'Wittgenstein the Man' and the exposition of his philosophy.

Phillips touches on an important and difficult tangle of problems when he claims that there is an "intimate link between [Wittgenstein's] life and his work", but he does not really attempt to clarify them. He relies very uncritically on sources concerning Wittgenstein's life which are less than impressive, such as W. W. Bartley III's *Wittgenstein*, some of whose extravagant claims are accepted as gospel, without any allusion to the surely justified doubts of many reviewers of that book about the quality of the evidence adduced in support of those claims. More importantly perhaps, he shows no signs of having reflected on any of the really puzzling questions about the relationship between a man's life and his work of the sort raised for instance, by Rush Rhees in his discussion of Bartley's book in *The Human World*. Yet those questions, one might think, have a direct bearing on the issues in the 'sociology of knowledge' which are at the centre of Phillips' interest.

I am not sure how useful the very compressed account of Wittgenstein's early and later philosophy will be to those to whom it is addressed. I doubt if it will be very intelligible to people who are not already pretty familiar with the issues involved and, though the presentation is superficially lucid, there are a number of more or less serious examples of carelessness of which the following are just examples. On page 30 it is said that, according to Wittgenstein's *Philosophical Investigations*, "Nothing exists outside of our language and actions which can be used to justify, for example, a statement's

truth or falsity". It is true that Wittgenstein opposed the view that there need or could be an "external" justification of the grammar of our language; and also that he thought this grammar required a certain "agreement in judgements"; but this does not mean that he denied the possibility of establishing the truth or falsity of any statement by reference to things which exist independently of us and our linguistic practices. Again, Phillips writes, on page 82, that according to Wittgenstein "it is a contingent fact that the concepts of pain and grief connect up with a certain uniformity of judgement among human beings". Clarity about what is contingent and what is not is of the essence in such issues: what Wittgenstein said is contingent is that there should exist the sort of "uniformity of judgement" among human beings which makes it possible for them to have concepts such as grief and pain; not that, given such uniformity of judgement, men's concepts of grief and pain connect up with it. I am afraid that slips of this sort, about quite fundamental issues, may seriously mislead the newcomers to Wittgenstein's philosophy to whom, I take it, the book is addressed.

Chapter 5 seems to me the best. It deals with claims about the incommensurability of scientific theories involving different "paradigms" arising out of the work of writers like Kuhn. Here, Phillips makes illuminating use of Wittgenstein's view that the language of scientific theorising has a continuing dependence on the language of everyday life; and he connects this usefully with Wittgenstein's discussion of the distinction between "seeing" and "seeing as" (or "seeing an aspect"). This chapter deserves attention by anyone interested in the questions surrounding the notion of incommensurability of theories. I hope readers will not be misled by Phillips' idiosyncratic use of the term "meta-language" in speaking of the relationship between the language of everyday life and the languages of the sciences. His conception has nothing in common with that expressed by the established technical use of "meta-language" amongst philosophers of language and logicians influenced by Tarski.

Chapter 7 has some useful material on the power structures of scientific institutions and their role in the "validation" of scientific theories. Phillips surely goes too far, however, when he claims that the individual scientist's "explanatory achievements are fully [my emphasis] dependent upon the judgements of the scientific community or the speciality area in which he works" (page 40). This would lead back



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to the type of relativism which earlier chapters were designed to avoid; and Phillips here forgets his own correct contention that the technical languages spoken by "scientific communities" are not fully autonomous, but depend for their sense on a relationship to the non-

technical language used in the wider human community to which they belong. A particular scientific elite is not a court beyond which there is no appeal. □

Peter Winch is Professor of Philosophy at King's College, University of London, UK.

## Remote and legendary figure

Sydney Selwyn

*Joseph Lister, 1827-1912.* By Richard B. Fisher. Pp. 351+16 plates. (Macdonald and Jane's: London, 1977.) £7.95.

ON the 150th anniversary of his birth and a mere 65 years after his death, Joseph Lister has already become an extremely remote, almost legendary figure. Even in his lifetime, during the 40 years of fame when every conceivable honour came his way, Lister appeared aloof and enigmatic. Little or no insight into his personality can be gained from the only two full-length biographies available until now, that of G. T. Wrench published in 1913, and the official one by Lister's nephew, Sir Rickman Godlee, which first appeared in 1917. Happily, the new biography by Richard Fisher deals fully not only with Lister's varied scientific and surgical work, viewed from the perspective of our own time, but also with Lister the man. Drawing on a considerable volume of unpublished correspondence, diaries and laboratory notebooks, the author's portrait will reveal for most readers an unexpectedly complex individual. Certainly, the popular view of Lister as the serene benefactor of mankind whose work on antiseptic surgery was greeted with universal acclaim has long needed correction. A glance through the editorial and correspondence columns of the *Lancet* and *British Medical Journal* from 1867 into the 1880s shows Lister to be at the centre of polemics—many of his own making and not all concerned with antiseptic surgery.

Lister was fortunate in his background—a congenial quaker family originating in Yorkshire, quaker friends such as Thomas Hodgkin and Elizabeth Fry who were devoted to science and philanthropy, and a prosperous wine merchant father, Joseph Jackson Lister, who by 1830 had made fundamental contributions to microscopy and was to become a life-long mentor and confidant of his son. As a student Lister performed extremely well at University

College, London until 1847, when he underwent a religious crisis and in March 1848 suffered a severe 'nervous breakdown' of a depressive nature, which prevented any further work for 18 months. Although he eventually made a good recovery, Lister subsequently was afflicted by recurrent self-doubts and a type of paranoia. His biographer convincingly uses psychological analysis to explain the paradox of a man who was gentle, kind, chivalrous and in many ways noble, but who was often publicly tactless, obtuse and, for example, an implacable opponent of the entry of women into medicine. Fortunately, Lister's mar-



Lister at Glasgow in 1865

riage, though childless, was extremely happy. His wife (a non-quaker) was his constant support, amanuensis and laboratory assistant. Moreover, in the stormy early days when Lister was under frequent attack in the medical journals he had the consolation of regular support from the young journal *Nature*.

Lister's scientific work is, in general, well reviewed. The early studies on involuntary muscle, inflammation and blood coagulation earned him, at the age of 33, a Fellowship of the Royal Society—where he joined his father and was eventually a most distinguished President. Most of this work was primarily carried out to provide material for Lister's undergraduate lectures. Using the microscope perfected by his father, Lister over a

ten-year period made meticulous observations on various contractile tissues and on the effects of irritants on the microcirculation of the frog's foot web. He greatly clarified the early events in inflammation by describing, in sequence, arteriolar constriction, slowing of blood flow and increased adhesiveness of erythrocytes—the latter having been first observed *in vitro* by his father and Thomas Hodgkin in 1827. Simultaneous changes in the pigmentation of the foot web underlined for Lister the truth of John Hunter's belief that inflammation was an active process. Similarly, blood coagulation was also shown to be a series of processes governed by tissue and vascular factors and not due, as was widely believed, to the passive loss of a 'liquefier' such as blood ammonia.

Lister always held that this work was his major scientific contribution: bacteria, which were so prominent in his later work, were simply specific irritants that triggered off inflammation and, occasionally, intravascular coagulation. To most modern observers, however, his pioneer microbiological researches from 1868 until the mid-1880s seem far more important. They were not only crucial in the battle against abiogenesis, but provided much-needed scientific support for Lister's 'Antiseptic System', which was at first extremely vulnerable at the hands of informed opponents.

Adequate attention is given in the book to Lister's technical achievements, including his method of obtaining in 1877 a pure bacterial culture by serial dilution. But the author in discussing the complex early studies on bacteria and fungi misses the extraordinary fact that Lister observed the antibacterial action of *Penicillium* species in 1871, and later used crude penicillin preparations in treatment.

Unfortunately, the book contains many medical and medical historical solecisms. For instance, jaundice is not synonymous with hepatitis, pin-worms do not cause skin disease ('ringworm' does), hospital gangrene was not due to soil-borne bacteria, and hydrocele is not a testicular growth. William Harvey published in the seventeenth, not the sixteenth century; Sir James Simpson worked in Edinburgh, not Aberdeen; Ehrlich's great chemotherapeutic work was on arsenicals, not mercurials; and Behring was certainly not a colleague of Pasteur. Although these and other blunders are irritating, Mr Fisher has written a definitive biography of a remarkable man. Such a book has been long awaited and is to be warmly welcomed. □

Sydney Selwyn is Reader in Medical Microbiology at Westminster Medical School, London, UK.



# American Nobel elite

Steven Rose

*Scientific Elite: Nobel Laureates in the United States.* By Harriet Zuckerman. Pp. xv+335. (Free Press: New York; Collier-Macmillan: West Drayton, UK, 1977.) £11.25.

NOBEL PRIZES are news and not merely because the money is of the order of a middle-sized pools win. The awards make not only *The Times* but the BBC news and *The Mirror* as well. National statistics of Nobel Prize winners are counted anxiously as a measure of scientific health. The Prize is seen as the apotheosis of a scientist's career, and the equality of opportunity with which graduate scientists are supposed to start up the hierarchical ladder emphasises the myth that, like napoleonic field-m Marshals, the laurels are in every Ph.D. student's lab-coat.

Nobel Prize winners are the nearest equivalent in science to archbishops, or pop or movie stars (barring the age-differential, of course, so far as the latter are concerned). They are invited

to serve on government committees in-differently, more-or-less, to the actual subject-matter of their expertise; the media hang on their words; films and TV plays are made about them. They pronounce with authority on subjects as diverse as ethics and ecology, the limits of human intellect and of industrial growth.

No appeal for humanitarian causes, whether a paid advertisement in the western press or a smuggled *samizdat* letter, is complete without the mystic *imprimatur*. Clearly, the phenomenon of the laureate is one of some importance in understanding the social system of science, whether considered from the point of view of the internal 'community' of scientists themselves or its relationship to the wider social matrix within which it is embedded and which it helps shape.

For the sociologist, I would guess that the problem of the study of elite groups must be considerable. Their numbers are small and individuals easily identified so that generalisations are harder; they are likely to be less amenable to standard procedures of interview or questionnaire than the less illustrious subjects who generally form the core of sociological specimens, and this must put the researcher's relationship with his or her subjects on a different (more deferential?) footing. Also, the objects of one's survey are actually likely to read what one writes—and if they don't like it, they have their ways. . . . Perhaps it is for this reason that the poor are more often studied than the rich?

Despite these problems, some of which she discusses, Harriet Zuckerman has been attempting, since the early 1960s, a dissection of the anatomy of the Nobel elite, or at least that not inconsiderable fraction of it which is resident in, and preferably born in, the US. She began by interviewing some 41 of the 56 laureates alive in the US in the early 1960s, and has subsequently extended her survey backwards to the first US laureate, and sideways to holders of what she calls the "41st chair"—scientists whose work is rated as of "Nobel quality" but, for varying reasons, were never awarded the Prize. Through some 200 pages of her book this tiny group are tabulated and cross-tabulated.

We learn that American laureates are more likely than the average researcher to come from an upper-class background (rating below supreme court justices, but above admirals and generals); more likely to be of Jewish and less likely to be of Catholic parentage (for complex reasons); more likely to have gone to elite colleges as undergraduates and especially as Ph.D.

students; and quite likely to have been chosen by, or opted to work with, a present or future laureate early in their career. They have written more papers, more often cited, than the generality of scientists or even other lesser-prestige groups, such as NAS members. They were promoted earlier to Chairs. They are likely to have done their prize-winning work at around 39 (older for the physiology or medicine prize, younger for the physics one) but only get the Prize some 12 years later. Half of the prizes have been won for work carried out in five US institutions.

Once they have received the Prize, their productivity tends to decline, but still remains high compared with other scientists. They accumulate many more honorific awards; they help construct new laureates; as they grow older, many turn towards philosophy.

There are a number of possible reactions to this careful compilation of data; for many professional scientists, it is likely to be an unsurprised "so what?", as they see so many of their rule-of-thumb categorisations confirmed, coupled with an enjoyable guessing game as they try to assign the unattributed quotes with which the Zuckerman text is enlivened. For the empirical sociologist of science, it is pleasing to see such a pretty working out of what Zuckerman's mentor, R. K. Merton, has called "The Matthew effect" in science—to him that hath, more shall be given; a maxim which clearly applies both to impending laureates and the institutions in which they work.

In addition, by showing convincingly that the Nobel award is neither a pure accolade of merit or part of a self-fulfilling prophecy, but is a combination of achieved and ascribed glory, Zuckerman has gone a little way towards demystifying the Prize. Laureates are neither born nor made, but are produced by one of those well known but little understood interactions. She has thrown up some further empirical 'puzzles', such as that of the religious background of laureates, and provided a basis for the comparative study of scientific and other elites (for instance, between science and literature Nobel laureates?).

One cannot help feeling, however, that this type of approach leaves many of the central questions untouched. The Prize glorifies the individual scientist, yet in an era of big science, where production, particularly of physics, has become virtually industrialised, most research has become teamwork. Teams cannot win Prizes—except, it seems, for peace. So, either they are unrewarded or some of their members are singled out—theoreticians rather than experimentalists, generally; professors

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## The CUBISM of IDEAS MANIFESTO

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It is self-evident that a theory of knowing Nature must have the same structure as Nature itself, but what is the "structure of Nature"? The recognition of this structure was delayed for these many centuries and millennia only by the late development of physics (particularly theoretical physics) and the lack of a parallel or integral dialectics. But at last, in the CUBISM of IDEAS, which I have by great good fortune discovered, we have the basic principle in hand!

### THE ACT OF REASON—

There is a blind spot in the dialectics of Aristotle, Hegel, Marx, Engels and Mao — "every idea implies an opposite idea" is only half the truth, every pair of ideas implies an opposite pair of ideas. Not two units but four *are necessary* to constitute the act of reason. In the constitution of light, we have magnetism (*bipolar*) and electricity (plus and minus) = 4 terms. In my theory of knowledge (also), ideas are quadratic, not dual!

The CUBISM of IDEAS is absolutely fundamental in all the arts and sciences. It also mediates between the physical and metaphysical worlds. It has deep roots in the soul or the religious instinct of man, which cannot be ignored in psychology — as Carl Jung demonstrated, and as depicted in the mystical scriptures and art of the great religious faiths, and even in primitive cosmologies.

It provides us with a faithful mirror of reality and at least a metaphor of ultimate truth. It connects physical, psychological, and spiritual reality thru their common fundamental structure — and therefore provides the foundation for a new psychology of the future, as well as a new art, which will give society its true and pre-destined dialectical unity.

Any perception is already dual, because every percept is viable only with reference to the background out of which it arises and from which it is distinguished. Even so it is only on the level of the nerve-sensor — commanding a reflex but no more, integrative thinking, which is the integration of percepts, is therefore necessarily at least quadratic, and all knowledge, whether conscious, unconscious or subconscious, is quadratic at base.

By raising and also limiting the dialectics to four numbers or dimensions, but maintaining a symmetry of 2/2 so that pairs can cross each other, I universalize the dialectics, and elevate it to equivalence with physical law. This is the double dialectics . . . the first and only basic advance in the dialectics in 3,000 years. The interpenetration of two pairs of ideas in the double dialectics brings the cubism of ideas into existence.

The CUBISM of IDEAS gives knowledge photonic form. If you want to believe that knowledge is light, then you must admit the existence of photons or quanta of reason.

Pairs of ideas fuse or disengage in these photons or quanta of reason. In doing so they gain or release energy. This energy is measured by the power of the ideas to influence our thoughts, not in "ergs". When idea-pairs unite, we imagine there is a spark — new knowledge always appears to us like a flash. This might be compared to the *photon* of light released when an *electron* jumps orbit, or is transformed to light by collision, thought is incandescent . . . it is illumination . . .

The photon of thought appears in memory as the "light of experience" or the "light of the past". Below the surface, it is the substance of dreams and intuitions.

The CUBISM of IDEAS controls the higher mental processes, permeates the subconscious, turns chaos perpetually into order, and forms the very fibre of our intuitions.

If not for dialectical control, we could not stand upright! And not only the mind, but the *entire* body (dancers may note) is a dialectical instrument.

Man is a part of Nature, with its wonderful waterfalls, and oceans, trembling mountains, rolling plains, shooting stars, and spiralling galaxies. But he is an intelligent part — and his thinking continually transforms the world. *Structural fitness* is one of the criteria of truth. *Are we fit for our environment?* Yes, for our natural environment. The degradation of the environment comes from the fact that we have not yet learned how to make our minds work as if Nature *herself* were directing our enterprises. Therefore, we must take the problem of the structure of knowledge seriously.

There will be many men in the future who will be able to use the new dialectics in grand leaps of the imagination and with fantastic effectiveness. It is the greatness of the theory of relativity that the formula holds good for all substitutions of co-ordinates. In the CUBISM of IDEAS, the structure holds good for all substitutions of terms or pairs of terms.

Einstein figured the physical universe as *pure geometry*. If we believe this to be true, we should try to utilize this geometry in our art. There is no other way in which this can be done but by the CUBISM of IDEAS. By this means our art will reflect at least a bit of the ultimate reality.

The CUBISM of IDEAS is as important for *modern art* as was the discovery of the "golden number" (or golden proportion) by the ancient Greeks. It is a philosophy of art face to face with elemental Nature — at the very sources of experience. Such an art shares with Nature her eternal qualities.

Disorder and chaos in our outer and inner worlds arises from errors of thought — the break with the universal. But it is not Nature that is awry, but we. If we put order into our thinking, we shall be able to restore order into our world.

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The CUBISM of IDEAS, 18pp. script, offset folio, unbound. \$5.00 (U.S.) per copy, by sea mail (air extra). CENTRUM MUNDI for EXPERIMENTAL DIALECTICS and the CUBISM of IDEAS, Morris Redman Spivack, Founder, Poste Restante, Reykjavik, Iceland.  
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rather than graduate students, generally; men rather than women, generally; qualified scientists rather than the technicians on whom their work depends, always.

The Prize typifies the myth of 'science' and obscures its reality. It emphasises the hierarchical and authoritarian shape which science in the late twentieth century has come to take. Its social functions inside science and in terms of the relationships between science and society, are both symbolic and substantive, and proper

matters, one would have thought, for a critical sociologist to discuss; yet in all of Zuckerman's pages scarcely a mention is made of such questions. Indeed, it has so far been left largely to scientists themselves to voice such doubts. It would be nice if, now she has done such a thorough piece of internalist empiricism, she would move on to these richer and more complex questions. □

Steven Rose is Professor of Biology at the Open University, Milton Keynes, UK.

## Dazzling firmament

Rainer Goldsmith

*The Pursuit of Nature.* By A. L. Hodgkin, A. F. Huxley, W. Feldberg, W. A. H. Rushton, R. A. Gregory and R. A. McCance. Pp. 180. (Cambridge University: London, New York and Cambridge, 1977.) £7.50.

PRY the poor reviewer, a mere mortal, confronted with a book of essays the authors of which are the living immortals of his discipline. Praise will be interpreted as sycophancy; criticism is tantamount to lèse-majesté. Yet the task must be done, and this fool will step in where wiser angels fear to tread.

The six essays, collectively entitled "Informal Essays on the History of Physiology" are mixed bags containing reminiscences, anecdotes, historical analyses, futuristic speculation, straightforward review, and personal prejudice. They make for varied reading; hardly ever boring, in part thought-provoking and often leaving the reader gasping with admiration at past achievements.

Outstanding among the six is the contribution of Feldberg, "Reminiscences of an Eye-Witness", being an account of the discovery and confirmation of the nature of synaptic and neuromuscular transmission. The lively and very personal style of the essay graphically reflects the enthusiasm and excitement of two or three highly creative years, interspersed by lobster dinners to celebrate successful new findings. One may wonder, however, if Feldberg really contributed only the key of eserine and the eserinated leech muscle to this remarkable period in physiology. Twenty-five years after the event, your reviewer began his scientific career in the very room in which these momentous advances were made: the ceiling was crumbling, the teak benches were uncommunicative witnesses of their past glory—just in one cupboard did the ghosts still linger, the shelves were marked Dale, Feldberg, Brown and McIntosh.

In general, the other essays are also best when the authors write about their own experiences or their immediate interests. The last quarter of Rushton's essay, the piece on colour vision, the *femme fatale* by whom he is so willingly seduced, is entertaining, informative and provocative, indicating that discoveries still await the lucky or the hardworking. The rest, though not up to the same standard, can hardly fail to please. Gregory's vivid account of a single afternoon's work which led to the discovery of secretin and the frustrating tale of the discovery, rejection and rediscovery of gastrin, are clearly very near to his heart.

McCance's contribution is a staccato, somewhat disjointed, review of perinatal physiology interspersed with acid comments on political matters, climaxed by an attack on ethical committees "which shackle the human physiologist to a politically-minded and usually uncooperative public". A



clearer explanation of what the physiologist is about would bring about greater cooperation.

What of the two brightest stars in this dazzling firmament?, the Nobel laureates: your reviewer is now skating on very thin ice. Sir Alan Hodgkin gives a dry, low-key, understated account of his own and other experiments which led to the explanation of the nature of the action potential. His picture of Cambridge in the 1930s is that of another world. But, with decreasing funds, we too may have "to think hard to decide what is right" and then "start in a bare room and build most of our equipment". The writing lacks Feldberg's sense of immediacy and involvement; it seems that life was an orderly procession from Cambridge to New York and the grand tour of the United States with just time for a less-than-one-dollar-a-day holiday in Mexico and then back to Cambridge and Plymouth. Great events are curiously muted: the tribulations of war are recalled by the fact that he did not bother to keep his copies of the *Journal of Physiology*, their alleviation by marriage to Peyton Rous' daughter, and his ability to think about nerves again; the 1947 fuel crisis during which all Cambridge and indeed the rest of the country froze, is marked only by the chance to do experiments at  $-4^{\circ}\text{C}$ . It is an account of a dedicated scientist hard at work undisturbed by counter-attractions—how unlike the more frivolous attitudes of a younger generation of Cambridge laureates (see J. D. Watson, *Double Helix*, Weidenfeld and Nicolson).

"Looking Back on Muscle" by A. F. Huxley is a masterly account of the changing views down the microscope over the past hundred years, which lead eventually to the sliding filament theory of the contraction of muscle. It is a stern warning that what is true today may be forgotten tomorrow and that yesterday's discoveries may be nearer the truth than today's dogma.

All in all, a stimulating book requiring a good physiological background to obtain full value. It is a pity that these eminent men allude to so much endowing us with their own immense understanding but in fact leaving most stranded on the shores of ignorance. It is as if the secrets must remain hidden from all save those who have been fully initiated into the circle. It is an enjoyable book for the student, opening the door into living history and introducing him to all the greats, not only these six but also their colleagues and their teachers who constitute the very backbone of physiology. □

Rainer Goldsmith is Professor of Physiology at Chelsea College, University of London, UK.

Hodgkin at Plymouth Marine Biological Establishment, 1963

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## Neurological traditions upheld

J. Z. Young

*Mechanics of the Mind*. By Colin Blakemore. Pp. 208. (Cambridge University: Cambridge, London and New York, 1977.) Hardback £10.50; paperback £3.95.

THE brain was the subject of the second series of Reith Lectures in November 1950. At that time I estimated that there might be perhaps 100 competent students of the brain in the world, as compared with at least 100,000 chemists. Since then, many of these latter have joined in the study of the brain; and Colin Blakemore, giving the lectures 27 years later, has to summarise the work of a very larger number of neuroscientists. He has performed the task magnificently and with the help of the Cambridge University Press produced a book of outstanding beauty as well as intellectual interest. There can be few works that contain so much that can stimulate the specialist as well as the layman. Perhaps it is one of the achievements of the Reith Lectures to effect this union. Producing them, one discovers that explaining things clearly is itself a road to discovery.

Blakemore shows this brilliantly in his treatment of the visual cortex, the part of the brain with which he is most familiar. He solves the problem of how to describe the complicated series of visual areas by calling them 'an amazing array of charts, ... a veritable atlas of the world of vision. Each map has a different scale and like the various pages in a real atlas ... the visual maps each emphasise a different component ... colour in one, shape in another ...'. And then, after talking of the 'torrent of information pouring through this labyrinth of maps' he brilliantly describes the effect of a local injury as 'causing a partial loss of the description of the world, a kind of scotoma of knowledge'.

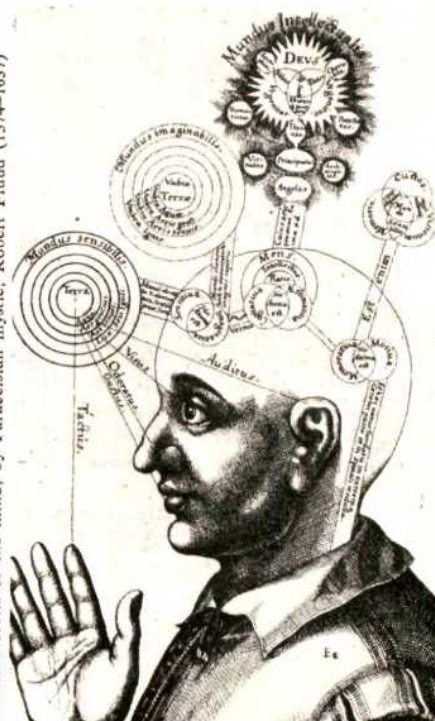
Blakemore is indeed not afraid to use imagery and he does it in a way that calls attention to the important problems—philosophical as well as neurological. 'We seem driven to say that such neurones have knowledge. They have intelligence, for they are able to estimate the probability of outside events'. It is interesting that he hardly ever feels the need to use inverted commas.

Anyone who wants to find out about the nervous system should read this

book. I will bet that even the most sophisticated neuroscientist or clinical neurologist will find something he did not know before. Apart from everything else he will find a great deal of history, some of it most ingeniously illustrated, sometimes in excellent colour. Personally, I could do without the photographs of distinguished neuroscientists; most of those that are of people I have known fail to capture their more characteristic expressions.

Of course, the treatment can be criticised here and there. Surely the surprising thing about knowledge of the pineal today is not that its function is 'still uncertain', but that we have learnt so much about it recently. Which reminds me that one of the things one misses is treatment of the neurochemistry of the brain. The index has no entry for amines (but four for animal spirits).

Any discussion of cerebral functions must include the controversial questions of nature and nurture, and the tabula rasa. Blakemore deals with them openly and in the main accurately. There is indeed much evidence that children are pre-programmed for language, but we anatomists are sceptical of the assertion that Weinicke's speech area is larger on the left side even in babies. How do you measure it? Blakemore gives more attention than I would to the hypothesis of specific memory molecules but comes down firmly against it, in favour of a 'transformation of the connectivity of the brain'. He refers to the 'built-in reflexes as the inherent knowledge of Plato', but curiously does not link discussion of this with his own discovery of changes of built-in capa-



Taken from *Utriusque Cosmi* (1619-1621)



cities that are brought on by experience. He rejects "the fantastic notion that every memory is innately within us", but his own evidence is consistent with the conception behind my mnemon hypothesis (which he quotes) that memory is a selective process, involving loss of some channels and improvement of others. Like all good neuroscientists he accepts that neural coding involves putting each item of information into a separate channel, but we all find it difficult to work out the im-

plications of this multichannel method of operating even for simple acts of perception.

To be able to discuss such questions in a review of the book of a series of broadcast lectures that are widely listened to is the highest tribute one can give to the author and to the BBC. Can any other country equal the record of this series? ☐

*J. Z. Young is Emeritus Professor of Anatomy at University College, London, UK.*

## Just not cricket

Stuart Sutherland

*Against Behaviourism: A Critique of Behavioural Science.* By Edmund Ions. Pp. xiii+165. (Blackwell: Oxford, 1977.) Hardback £6; paperback £2.75.

THE sport of debunking famous names rarely fails to entertain, and when applied to men as pompous and pre-tenacious as most social scientists it has enhanced spectator appeal. As a demon bowler Edmund Ions is in a class of his own: he dismisses in successive balls Herbert Simon, Morton Kaplan, Karl Deutsch, B. F. Skinner, Rashevsky, Stouffer, Adorno, von Neumann, Kaldor, Hicks, Garfinkel, Goffman, Levi-Strauss and Chomsky. His opponents are a curiously assorted team: were it not for the inclusion of Garfinkel and Goffman one might suppose that Ions was combating the attempt to apply objective methods in the social sciences, but ethnomethodologists can scarcely be accused of that and are declared out for giving "a partial and selective account of human behaviour".

*Against Behaviourism* is primarily an attack on the use of mathematical and logical theories, and of scientific methods of data collection and analysis in the social sciences. Ions argues his case by examining a series of instances where the attempt to be scientific has resulted in banal or useless theories, or where objective methods of data collection have been poorly applied and grossly misinterpreted.

There is plenty of bad work in the social sciences and many of Ions' examples are convincing. Grandiose models of the sort constructed by Rashevsky are empty; cost-benefit analysis invariably involves making arbitrary assumptions; the celebrated discovery of the "Authoritarian Personality" rests on the construction of a loaded questionnaire and the use of a monstrously biased sample; Games Theory is an idealised model of conflict

behaviour; and cliometricians, jurimetricians, psephologists and content analysts often either discover the obvious or make wild errors as a result of unduly restricting both the data they use and their methods of analysis.

Moreover, Ions is surely right in thinking that the very obscurity of many scientific approaches to the social sciences has disguised the fact that much of the work is empty. Model builders often conceal the banality of their thought by the tortuousness of their language. It requires both perspicacity and courage to dismiss as trivial such statements as: "*Hypothesis Two*. Multiple role functions operate on personality systems in such a fashion that the personality systems perceive the objective and values of the decision making units linked by the multiple role functions as more similar than they would if they entered only into single role function." (Morton Kaplan.)

We may agree with Ions, then, that the application of scientific or objective methods is no guarantee of producing interesting findings or arriving at correct conclusions, but he seems to go beyond this and to damn all attempts at objectivity. Yet, the use of intuition can be just as fallible as is demonstrated by the results of studies on the unreliability of unstructured interviews for job selection. One only has to read Arthur Maslow to become aware that the experiential or humanist approach to society may itself result in pretentious banality and silliness. And even if rigorous models are as incomplete as non-rigorous ones, they may still throw light on human behaviour by focusing attention on the ways in which people depart from them.

Ions stigmatises Levi-Strauss for "classifying", though how it is possible to communicate at all without classifying he does not explain. His critique of Chomsky is hard to follow. He seems to think that the attempt to formalise our intuitions about syntax is in itself a bad thing and then goes on to criticise Chomsky for failing to deal with those aspects of language that are completely outside the scope of his theories.

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A further problem is that in arguing from example, Ions is himself guilty of one of the main crimes of which he seeks to convict his opponents, namely, dogmatic generalisation based on insufficient evidence. He maintains a consistently fast pace but his bowling is loose and often aimed at the man

not the wicket: any decent umpire would have sent him off the field, but that would have deprived the crowd of considerable amusement. □

*Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex, UK.*

## Animal communication in ethological research

John Krebs

*The Behaviour of Communicating: An Ethological Approach.* By W. John Smith. Pp. 545. (Harvard University: Cambridge, Massachusetts and London, 1977.) £13.65.

MORE than any other subject, animal communication has occupied a prominent place in ethological research throughout the past forty years. Lorenz's demonstration that displays of ducks and geese can be used as species-specific taxonomic characters to trace phylogenies; Tinbergen's classic analyses of the motivational basis of displays in gulls; Thorpe and Marler's work on the ontogeny of bird song; and von Frisch's dancing bees are all justifiably recognised as pillars of ethology. In the past few years, although some of the emphasis has slightly changed, animal communication has still played a central role in ethological thinking, exemplified by G. A. Parker and J. Maynard Smith's application of game theory to ritualised displays.

It is perhaps not surprising, in view of the voluminous and diverse literature, that no single person has, since Tinbergen with his *Social Behaviour in Animals* (Methuen, 1953), attempted the daunting task of reviewing the whole subject of communication. There have been notable collections of essays (Huxley's "ritualisation" symposium, Hinde's *Non-Verbal Communication* and Sebeok's *Animal Communication*), but W. John Smith is the first for nearly 25 years to write a comprehensive treatise. Comprehensive is the word: with over forty pages of references, Smith has done an admirable and scholarly job of bringing together the literature up to and including 1976.

Smith's own well known contribu-

tion to the field of communication has been to emphasise the distinction between the *message* contained in a signal (what it might potentially reveal about the communicator) and its *meaning* to the recipient(s). The meaning, measured by the response of the recipient, usually depends on the context. For example, a hypothetical call given by a bird whenever it moves may mean "I am about to attack" in one context, and "I am about to feed" in another. The book leans heavily on Smith's message-meaning distinction: after an introduction in which he clearly defines communication and states the problems he is going to tackle, there are six chapters (including a lot of descriptive examples) dealing with the structure and message content of signals.

The subsequent chapters deal in turn with the motivational basis of displays (a review of the traditional ethological work), the importance of context and the meaning of signals, ritualisation, ecological and other constraints on signalling systems, and finally a critique and reassessment of the display concept. In this final section, Smith points out that the usual preoccupation of ethologists with simple stereotyped displays (releasers) has led to an under-emphasis on the possibility of complex grammatical rules for combining simple components into complex signals. Throughout the book, Smith provides numerous examples, many of them unpublished, which make interesting reading and act as an excellent diluent for the slightly heavy style of the more theoretical discussions.

In such a wide-ranging and thought-provoking book, it is hard to know where to begin in singling out points for discussion, but I will mention briefly a general feature of communication which I believe that Smith may have underplayed. Smith views communication as a cooperative venture between communicator and recipient; displays are acts "specialised to make information available to the recipient" (p69, p195) about the communicator's likely future behaviour, status, location, and so on. This standpoint is emphasised by Smith's statement that manipulation or intentional misleading is a "disturbing possibility" but not one that ethologists have yet discovered. (He inexplicably separates intra- and interspecific deceit—the

latter is well known in, for example, Batesian mimicry.)

I would suggest that far from being a marginally possible aberrance, manipulation, or something very like it, is a central feature of much of animal communication. As Smith emphasises, signals can only evolve if there is an advantage to the recipient in responding, as well as an advantage to the communicator. But this is not to say that the benefits to the two are equal; and it seems inevitable that both participants in any interaction—be it between a territorial stickleback displaying to an intruder or a fledgling blackbird begging from its parent—will strive to get the maximum benefit. Viewed in this light, communicators are always trying to manipulate or persuade recipients, while recipients are increasing their sales-resistance. It is then not at all surprising that displays often provide incomplete information about the motivational state of the communicator (p201): it always pays to be poker-faced when selling a used car.

It also follows from my argument that many display interactions are concerned with assessment ("Is he really as confident as his threat indicates?"). The elaborate courtship rituals of many animals may have as much to do with assessment of the potential mate's fidelity and fecundity as with "helping male and female to cooperate in beginning copulation" (p300). This was brought home to me by the striking result of Erickson and Zenone (*Science*, **192**, 1353–54; 1976). They showed that a male ring dove actually rejects a female who is too willing in courtship. This is a canny reaction: "precocious" females are forward because they have just been stimulated by mating with another male, and it supports the view that ring dove courtship involves assessment by the male of whether the female has already been fertilised. With the traditional ethological view of male courtship as a means of arousing the female to copulate, who would have foreseen that male ring doves would reject avian nymphomaniacs?

While I certainly would not claim that all intraspecific communication involves manipulation and assessment, it just might be useful to bear these ideas in mind when analysing courtship and threat signals. Finally, let me emphasise that Smith's omission of the sort of discussion I have briefly outlined does not materially detract from his enormous achievement in synthesising and appraising such an important subject. □

*John Krebs is Lecturer in Zoology at the Edward Grey Institute of Field Ornithology, University of Oxford, UK.*

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# Expression of the genetic apparatus

John Scaife

*Gene Expression*. Vol. 3: Plasmids and Phages. By Benjamin Lewin. Pp.942/928. (Wiley: London and New York, 1977.) Hardback £24; \$40.65; paperback £10.25; \$19.50.

READERS of this journal will be familiar with the changing fortunes of microbial genetics in recent years. In the early to mid-1960s, its practitioners saw the genetic code unveiled and the birth of the operon. Five subsequent years of plenty were followed by a decline, as interest turned from lower to higher organisms and their development. Now the wheel has come full circle as the potential of bacteria and their viruses in genetic engineering is understood. As if to mark this development, the new volume in Benjamin Lewin's series on *Gene Expression* is devoted to plasmids and phages. These genetic elements have made a massive contribution to our understanding of the mechanism of heredity at the molecular level. They also happen to be suitable vectors of recombinant DNA made *in vitro*.

This series of books is intended to provide a critical analysis of the organisation and expression of the genetic apparatus at the molecular level. In this context, the present volume gives an excellent entry to the literature for advanced students of molecular genetics. It will also be a valuable reference work for final-year undergraduates. Rather than write a textbook, the author has manfully undertaken to review the whole field in some detail. The enterprise has been a profitable one. Indeed, his account of some systems is more incisive and digestible than the available specialist reviews. Readers will particularly appreciate the presentation of experimental protocols and models in diagram form, although grey rather than a brighter second colour can lead to ambiguity in some figures. Taken as a whole, the book with only a few important inaccuracies brings the general reader painlessly up to date in a fast-moving field.

Plasmids are now known to occur naturally in bacteria as widely different as *Staphylococcus* and *Agrobacterium*. The first of its kind to be discovered was the fertility factor F, which promotes mating between strains of *Escherichia coli* and, as it turns out,

other related enteric bacteria. It is a circular DNA molecule which, although much smaller (~2%) than the bacterial chromosome, is big enough to carry nearly 100 genes (94.5 kilobases). It can exist as an autonomous element but may insert into the chromosome.

Plasmids can vary in a number of respects. Some are unable to insert in the chromosome, others cannot mediate their own transfer by mating. They can carry genes of considerable importance in agriculture and medicine. For example, R factors are plasmids which confer multiple drug resistance on the host bacterium. Pathogens containing R factors have become a serious clinical problem, particularly since they can often transfer multiple drug resistance to other bacteria. Other plasmids determine the ability of some soil bacteria to utilise unusual materials such as camphor and toluene. At the same time, plasmids interest the molecular biologist as excellent tools to study, for instance, DNA replication *in vivo*, since they may be regarded and, more importantly, manipulated as dispensable analogues of the chromosome itself.

The bacteriophages form two large groups. The virulent phages are obliged to multiply on infecting their host. Most of them kill the host in the process. The important virulent phages of *E. coli* are discussed in this book, including the double-stranded DNA phages T4, T3 and T7, the single-stranded DNA phages  $\lambda$ X174, S13, f1 and M13, and the RNA phages f2 and Q $\beta$ . The temperate phages do not always kill the host. On infection they may enter the prophage state to give a lysogenic bacterium, in which the phage DNA is preserved as part of the host's hereditary material. There are temperate phages which are directly important to man, such as the corynebacteriophage  $\beta$ , which determines the toxin of diphtheria. Certainly the most studied of this group is phage  $\lambda$ , which is discussed in great detail in this work. Some readers will regret the omission of the phages of *Bacillus subtilis* and *Salmonella typhimurium* from this book. I was surprised to find the coliphage P1 given short shrift. P1 has the distinction of existing in the autonomous state as a prophage, qualifying for inclusion as both plasmid and phage. It is also an important vector of host DNA in genetic analysis by transduction, without which fine structure genetic mapping would be impossible.

The book devotes separate chapters to the different genetic elements, a profitable treatment for readers at the research level. A fuller index would probably have made it more acceptable to the general reader. The two opening

chapters describe the transfer material between bacteria, as naked DNA in transformation, and by mating (conjugation) between bacteria. These chapters provide a good account of the techniques of genetic mapping, and of the principles and mechanisms involved. They provide the background for later plasmid and phage chapters, and include discussion of the F factor. A separate chapter on plasmids focuses on R factors and col factors (which determine macromolecular antibiotics called colicins).

The molecular genetics of plasmids is examined in some detail. The mechanisms controlling plasmid copy number, transfer functions of F and related R factors, the molecular structure of plasmids as deduced from heteroduplex mapping techniques and the incompatibility between related plasmids in the same host are discussed in these early chapters. They also provide an informative account of studies on insertion sequences. These are specific DNA sequences, approximately 1,000 base pairs long, and are found in plasmids and at various sites on the *E. coli* chromosome. Interaction between insertion sequences can mediate insertion of F into the chromosome. Of perhaps even greater importance is the discovery that some R factor genes for drug resistance lie between insertion sequences and are transposed by them to new phages, plasmids and chromosomal sites. These movable genetic packages, now called transposons, provide a means by which drug resistance and other properties may reassort in nature.

The phage  $\lambda$  opts for lysogeny or growth shortly after infection. The molecular nature of this decision is not completely understood but it clearly depends on competition between mutually exclusive patterns of transcription. Of major importance in this competition are the factors facilitating or denying to the host RNA polymerase access to phage promoters. The disentanglement of these regulatory circuits by mutants, sequencing and other approaches makes a fascinating story. Dr Lewin's account of its development is one of the best currently in print. The virulent phages, T7 and T3, which are similar in size to  $\lambda$ , probably have simpler circuits. They code for an RNA polymerase of their own, and their growth is regulated at the level of transcription. The RNA phages, on the other hand, have important controls operating at the level of translation. The genetic organisation of RNA phages has rarely been elucidated by direct sequencing, since these phages do not recombine.

The integration of  $\lambda$  prophage into the host chromosome provides a

beautiful example of site-specific recombination, which may serve as a model for the movement of transposons and analogous elements in higher organisms. The interaction uses enzymes which recognise different, specific, base sequences (recently elucidated by Landy and Ross, *Science*, **197**, 1147; 1977) in the phage and host chromosomes, and promote crossovers between them. Most recombination, of course, is not limited to short sequences. Generalised recombination occurs between parental molecules of all phages but those with RNA as their genetic material. Models for the different types of recombination are discussed by the author in several chapters; and he gives a fair account of the current ideas in a changing field.

Most of the information encoded in a phage genome relates to the synthesis of the phage coat. More than 50 genes of phage T4 have this function. Notably, in phages T4 and  $\lambda$ , the assembly pathways have been mapped out by recording the stages reached in different maturation mutants. These

systems provide a way of studying spontaneous self-assembly and structural interactions between repeating subunits. They also lead to the conclusion that *in vivo* assembly is not spontaneous but is assisted by special accessory proteins. This is an area where Lewin's extensive diagrams and plates are most helpful.

*Plasmids and Phages* encompasses a wide range of modern techniques and ideas. They are described simply and well; and this presentation shows the dense logical and experimental substructure from which molecular genetics has grown. In addition, the author provides an excellent account of the early development of the major themes which continue to engage interest in the field. It is therefore fair to say that this book is valuable, not only as a research text but as a source which should be of considerable value to the future historian of molecular biology.

John Scaife is Reader in Molecular Biology at the University of Edinburgh, UK.

## Genetic engineering journalese

R. J. C. Harris

*Biohazard*. By Michael Rogers. Pp. 209. (Knopf: New York, 1977.) \$8.95.

THE title of the book does not alas describe it. What purported to be a description, in journalist's language, of the biohazard problems was a totally different book. The blurb of the cover outlines both the contents and the style: "Immediate, fascinating, frightening in its implications, *Biohazard* is the story of a current and major scientific controversy". The writer, Michael Rogers, portrays a "moral and scientific confrontation" reported "as it happened and where it happened".

Rogers outlines, and devotes most space to, the "history-in-the-making" meeting at Asilomar in early 1975 (pp51-100). He began, too, without an understanding of recombinant DNA; for him, then, the calculated breeding of plants and animals by selection and mutation was, in essence, genetic engineering. In chapters 2 and 3, we are led through the history of DNA (starting with Miescher and ending with Avery *et al.*) and acquainted with *Escherichia coli* and phages. The three chapters on Asilomar are preceded by

a recounting of the moratorium. Paul Berg enters the story with his dilemma about SV40 but fails to get the brief description accorded to most of the actors. Herbert Boyer, a soft-spoken San Francisco biochemist, discovered *EcoRI* which produced the "sticky ends" that allowed not only the original DNA strands to rejoin, but the coupling of the cut-ends of foreign DNA.

It is not clear how excerpts from the Asilomar meeting were reproduced with the apparently original comment of selected delegates. The taped official record was (p54) to be held for 50 years (*sic*). Did Rogers have his own tape, did he evade the 50 year rule, or had he merely adequate shorthand? In any event, this poses a general problem. Most scientists, even the most glib, prefer, if they are to be quoted verbatim, to see what they are purported to have said before it is published. Is it not courteous to ask the prior consent of the scientist before attributing to him remarks of any kind? These steps may have been taken for the US contingent where their scientists were usually quoted—for example, David Baltimore ("trimly bearded and clad in an embroidered Levi shirt") and Ray Curtiss ("imposing, long-haired") but not, probably fortunately, for most of the British. One who may recognise himself talks about K12 ("nice, quiet, boring person at least as far as his colon is concerned"). Two Britons at Asilomar are quoted (or misquoted) by name—E. S. Anderson from Colindale ("portly, imposing gentleman") and

Sydney Brenner ("bushy eyebrows, gleaming eyes, non-stop animation, mid-way between leprechaun and gnome"). One group, at least, was missing: those whose experience was in the realms of infectious diseases. Anderson drew careful attention to this (p63) and "glared coldly" as he did so.

Summing-up the meeting (at which some 10% were journalists, anyway) is well described. The problem is so acute—more akin to asking a committee to write a poem—that the most recent US meetings on recombinant DNA have compromised the organisers by drafting a statement (a letter in this case) and seeking adherents from among the meeting. Although Asilomar was attended by many microbiologists from different countries, only the British were singled out and the Russians are mentioned once.

The single chapter devoted to a discussion of biohazards begins with: "the only recorded civilian biohazard fatalities—the deaths of individuals who were in no way connected to a laboratory—occurred in London, in March 1973" and refers, of course, to the smallpox incident. Rogers uses this accident (albeit from a well-known human pathogen) to justify the widely felt concern about "new infectious organisms containing novel genetic material". Prominent among the concerned was, of course, Mayor A. E. Velluci of Cambridge, Massachusetts. The possibility (Velluci's "disease that can't be cured—even a monster") of a public, or ecological, disaster added bright new ammunition for the "town" in its confrontation with "gown". The author describes in some detail Building 41 at the National Institutes of Health at Maryland, which was built apparently for \$3.5 million, to handle the viruses which were expected by the US to emerge from the accelerated Cancer Program. The methods of handling biohazards, in genetic engineering research, might well have been stressed at greater length. In the UK, the Biological Safety Officers and their Safety Committees exist solely to assure the public (in the final shape of the Health and Safety Executive) that the containment level selected, whether this be physical or physical/biological, matches the risk and is thus safe.

The next chapter diverges from the story of succeeding conferences at which Rogers is becoming more confident, and more of a *confident*; he reverts to restriction enzymes, gel electrophoresis, complementary DNA sequences, molecular cloning and shotgunning. A crucial issue which is mentioned as a possibility is that of "libraries of cloned gene sequences" whether these be of prokaryotic (E.

## Posture

**Robert Roaf**

December 1977, viii + 100pp., £3.80/\$7.50 0.12.589350.7

Reference is frequently made to 'good' or 'bad' posture, but the criteria are seldom defined. The term 'posture' itself is difficult to define, because without a qualifying adjective it has only half a meaning. It has been defined as the relative disposition of the limbs and body, and as an expression of the body's equilibrium and balance. Professor Roaf considers the criteria for good posture to be balanced movement and minimum muscular exertion in adapting to the needs of the moment. He draws attention to the many diverse areas in which posture is significant — in cultural and sporting activities, and in communication between individuals and groups — and he discusses the origin and development of the upright position in humans, the physiological mechanism of postural control, and the causes of poor posture and its remedies.

## Group Theory and Computation

edited by **Michael P. J. Curran**

December 1977, xiv + 118pp., £5.80/\$11.35 0.12.200150.8

This volume is based on the invited papers given at a Summer School on Group Theory and Computation held under the auspices of the National Committee for Mathematics of the Royal Irish Academy. Together, they present in a concise form both the state of the art in certain areas of finite group theory and also a review of permutation groups.

### Contents

*Marshall Hall Jr.*: Computers in Group Theory. *John Leech*: Computer Proof of Relations in Groups. *J. H. Conway*: The Miracle Octad Generator. *J. H. Conway*: A Quaternionic Construction for the Rudvalis Group. *Peter M. Neumann*: Finite Permutation Groups, Edge-Coloured Graphs and Matrices.

## APIC Studies in Data Processing No. 13

## Software Engineering

**R. H. Perrot**

December 1977, xii + 204pp., £8.20/\$16.00 0.12.551450.6

This book records the papers presented at the British Computer Society's Symposium on Software Engineering held at the Queen's University of Belfast in April 1976. It defines the working tools of the software engineer, reviews the range of methods presently available to him and suggests further improvements. The publication is devoted to practical measures to alleviate problems in the use of currently available software engineering tools and it has an optimistic and forward looking approach. For the present, the primary consideration is how to live with existing software products and to use them more effectively. Several well engineered systems are examined and a study made of the lessons to be learnt from them.

## European Monographs in Social Psychology 12

Series editor: **H. Tajfel**

## Decoding Oral Language

**Astri Heen Wold**

January/February 1977, approx. 250pp., £8.50/\$16.50 0.12.336250.4

This book is the result of a long programme of theoretical enquiries and experiments on aspects of time in language and communication. Its emphasis on the temporal dimension in psycho-linguistic analysis makes a sharp contrast with current trends in Chomskian and post-Chomskian semantic theory, which have tended to *spatialize* and *deterioralize* language. A linguistic theory which treats as irrelevant conditions such as memory limitations, distractions, errors and shifts of interest, argues the author, cannot hope to come to terms with the realities of language as a series of intentioned acts of communication between one speaker and another. This study accordingly attempts to combine a soundly-based *social-psychological* perspective on language with an analysis of *individual processing*.

## Studies in Convection

Theory, Measurement and Applications

Volume 2

edited by **B. E. Launder**

November 1977, viii + 224pp., £8.80/\$17.25 0.12.428002.6

This series aims to present major contributions to the understanding of convective transport phenomena; particularly in turbulent flows. This, the second volume in the series, is devoted entirely to various aspects of convection relating to chemical reaction; in recognition of the growth and expanding interest in combustion research generated by worldwide concern about pollution and the energy crisis. Combustion, as an area of research, has benefited from progress made in the study of non-reacting flows, which has engendered new methods of analysis, new instrumentation and a general understanding of turbulence.

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## Measles Virus and Its Biology

K. S. Fraser and S. J. Martin

December 1977, approx. 250pp., £9.80/\$19.25 0.12.265350.5

This first book in the new series, *Experimental Virology*, is at the forefront of research on the measles virus, yet its contents are still within the grasp of the students of medicine, microbiology and biochemistry. The work sets out the problems of measles and its complications and relates them to recent knowledge of the biological character of the virus. The authors have placed particular emphasis on the predilection of the virus for lymphoid tissue and its ability to set up persistent infection through defective modes of replication. They have stressed too the need to find out more about the relationship between measles virus and immune responses. The significance of such interactions can be seen clearly in the pathogenesis of conditions such as measles enteropathy, subcutaneous sclerosing panencephalitis, multiple sclerosis and certain auto-immune diseases.

## Microbial Ecology of the Gut

edited by R. T. J. Clarke and T. Bauchop

December 1977, xviii + 410pp., £13.50/\$26.50 0.12.175550.9

This book sets out, by contrast, to provide a full and integrated account of the microbial ecology of many different animals. The multi-disciplinary origin of the contributions suggested the adoption of a broad rubric rather than separate specific studies: information has been organized within a framework of general questions — what kinds and numbers of organisms are present? What are their activities, and to what extent are these activities performed? Thus the book includes discussions on the bio-chemical activities of microbes in the gut, and fermentations in the hindgut and foregut. Other chapters deal with types of microbes; techniques of study; a gnotobiotic approach to ecological studies; protozoa in the rumen; and a mathematical approach to the turnover of fermentation end-products.

## The Analysis of Organic Materials An International Series of Monographs

Series editors: R. Belcher and D. M. W. Anderson

## Instrumental Organic Elemental Analysis

edited by R. Belcher

December 1977, xii + 300pp., £14.00/\$27.35 0.12.085950.5

During the last fifteen years automated elemental organic analysis has expanded to such an extent that it has almost superseded manual operations, for certain elements at least. The purpose of this book is to bring together the accumulated knowledge and experience of investigators who have been dealing first hand with these new methods. To provide some flexibility, two different commercial apparatus for the determination of carbon, hydrogen, nitrogen and oxygen, have been described — the Perkin-Elmer and the Carlo-Erba apparatus. A comprehensive account of the applications of the Dohrmann coulometric apparatus has been included and also the remarkably efficient Merz-Dumas method. The latter is extraordinarily versatile in its applications, for it can cope with trace amounts of nitrogen as well as high percentages.

## Oxalic Acid in Biology and Medicine

A. Hodgkinson

December 1977, xiv + 360pp., £15.80/\$30.25 0.12.351750.8

This comprehensive review of oxalic acid in living systems brings together for the first time work in several fields, including analytical chemistry, microbiology, plant physiology, toxicology, nutrition and human pathology. The book is intended to be a source of reference as well as a guide to current research. Dr. Hodgkinson traces the role that oxalic acid has played in the development of early chemical theory, describes the structure and properties of the acid, and examines the problems of determining oxalate in biological material. A variety of topics of current interest is covered, including the role of oxalic acid in plant metabolism, the growing problem of livestock poisoning by oxalate-containing plants, and the problem of human poisoning by agents such as xylitol and methoxyflurane.

## Annual Reports on NMR Spectroscopy Volumes 6C and 7

Volume 6C edited by E. F. Mooney

December 1977, xiv + 658pp., £33.50/\$65.50 0.12.595347.9

Volume 7 edited by G. A. Webb

November 1977, x + 300pp., £16.00/\$31.25 0.12.505307.X

Over recent years no other technique has grown to such importance as that of NMR spectroscopy. It has applications in all spheres of science concerned with the electronic and molecular structures of materials as well as those areas concerned with the reactivity of molecules in solution. Progress has occurred in both the experimental and theoretical aspects of NMR. These are reviewed in this series and presented in a manner suitable for chemists from all disciplines. *Annual Reports on NMR Spectroscopy* is well established as a medium for communicating recent progress in NMR and its applications to the specialist and non-specialist alike. The series is a vital source of information for workers in all branches of chemistry; including biochemistry and pharmaceuticals, and will continue to be a valuable source of information for all analytical, structural and physical chemists.

### Contents of Volume 6C

P. W. Hickmott, Michael Cais and A. Modiano: NMR data on organic-metal carbonyl complexes (1965-1971)

### Contents of Volume 7

R. Fields: Fluorine-19 NMR spectroscopy of fluoroalkyl and fluoroaryl derivatives of transition metals, M. Witanowski, L. Stefaniak and G. A. Webb: Nitrogen NMR spectroscopy. Roderick E. Wasylishen: Spin-spin coupling between carbon-13 and the first row nuclei.

## Monographs of the Physiological Society No. 34

**Corticospinal Neurones**

## Their role in movement

C. G. Phillips and R. Porter

December 1977, xii + 448pp., £16.80/\$32.35 0.12.553950.9

This book is more than just a compilation of experimental findings. By discussing their personal observations in the context of accumulated knowledge the authors have produced a classic work which will endure long after new research has outdated parts of its findings. It reviews and analyses all major work on the corticospinal tract, starting with extensive historical material and terminating with newly published work. The strength of this analysis derives from one or other of the two authors having been personally engaged in most of the types of work described.

## London Mathematical Society Monographs No. 10

Series editors: P. M. Cohn and G. E. H. Reuter

**Topos Theory**

P. T. Johnstone

December 1977, xxiv + 367pp., £17.50/\$34.25 0.12.387850.0

Since the pioneering work of F. W. Lawvere and M. Tierney in 1969 and 1970, topos theory has been one of the major growth areas of mathematics: in particular, it has been characterized by the development of striking new links between algebra, geometry and logic, whereby the methods used in each of these areas may be applied to problems in the others. This book is largely based on lectures and seminars given by the author at the Universities of Cambridge, Liverpool and Chicago, and is the first comprehensive account of these developments to be published.

**Surface Carbohydrates of the Prokaryotic Cell**

edited by I. W. Sutherland

December 1977, x + 472pp., £19.50/\$38.00 0.12.677850.7

This publication provides a current view of the composition, biosynthesis and control of the surface carbohydrates of prokaryotes, superseding earlier reviews in this rapidly expanding field. These aspects are related to the possible functions of the polymers and to their role as receptors for bacteriophage and as antigens or haptens. Enzymes degrading the polysaccharides are also considered and the information they provide about polysaccharide structure is discussed. The author indicates the direction of current research in the field, problem areas, and recent improvements in technique. *Surface Carbohydrates of the Prokaryotic Cell* will be of primary value to undergraduates, research students and workers in microbiology, biochemistry and related fields.

**A World Geography of Human Diseases**

edited by G. Melwyn Howe

December 1977, xxx + 622pp., £24.00/\$46.90 0.12.357150.2

Professor Howe's book deals with the main diseases of mankind on a global basis, bringing together a group of leading experts whose approach is varied and multi-disciplinary, but who share a particular interest in the problems of man's interaction with his environment, and in the global variability and spatial patterning of disease. Suffering and death have a multitude of different causes, but the diseases selected for this volume certainly account for a major share of the world's sixty million deaths annually. The text demonstrates the intricate links between physical, biological, socio-cultural and lifestyle hazards and the different causes of illness and mortality.

**Kidney Hormones**

## Volume II Erythropoietin

edited by J. W. Fisher

December 1977, xvi + 602pp., £24.00/\$46.90 0.12.257652.7

This second volume of this three part series on kidney hormones deals exclusively with the production of erythropoietin and the nature of erythropoietin itself, a renal hormone that has been clearly identified and characterised only during the past 10-15 years. Many aspects of erythropoietin are covered: the history of the development of erythropoietin and humoral control of erythropoiesis; physico-chemical characterization and isolation procedures for erythropoietin; pharmacology and physiology of erythropoietin; erythropoietin production in disease states in man; and the implications of erythropoietin for clinical usage.

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*coli*) or eukaryotic origin (such as yeast, frog or *Drosophila*). The UK Genetic Manipulation Advisory Group (GMAG) is currently insisting that all unrecognised DNA fractions from shotgun experiments should be destroyed rather than preserved in collections, as is apparently the case for such libraries in other countries. This seems to underline the necessity of seeking international agreement, as indeed, ICSU, ESF, EMBO and WHO are trying to work towards on issues such as these. There can never be a case for seeking a general code of conduct for handling genetic recombination experiments, but the *principles* could well be agreed. Among these is the amassing

that might be discovered. For industry in the UK and even for academic workers, there is, however, a strong disinclination to declare plans and objectives to GMAG in the detail required. How this problem of confidential treatment will be resolved remains to be seen.

Sydney Brenner, who figured prominently in the Asilomar discussions—often apparently coming to the rescue when a particular meeting seemed to have been stalled—seizes an opportunity to advance the concept of a “disarmed bug”—biological containment to supplement physical containment which Paul Berg (p151) had condemned in a letter, as “overrated and,

they will even try to understand all the issues at stake. Will it be enough that the UK watchdog, the Health and Safety Executive, reinforces the advisory group (GMAG)? The public does, indeed, have four representatives on GMAG together with ASTMS, IPCS and TUC union members.

Public opinion may well be satisfied in the UK that laboratory manipulations are carried out safely in inspected premises (for the two higher containment categories). Are the US public so satisfied? No, they are not. According to Rogers, they do not trust the scientists, judging them to be mere self-servers, anxious to gain instant recognition and credit at the expense of the public and, moreover, encouraged and supported by the NIH which is largely composed of a peer group with identical motivation. Normally, the older generation is more conservative than the younger. This issue has converted the younger in the US into “nay-sayers”. The “yay-sayers” are their elders.

The scientific debate has elicited a response from the law-makers in the US and a counter-response from the scientists. It seemed, until recently, that a Federal law would be passed regulating the issues. The scientific community does, however, seem to be gaining the upper hand. If this opens the way for States to enact their own rules, Mayor Velluci for one will, no doubt, be satisfied. There will be some States who will be strict and some not so strict. In which will the experiments tend to get done? There is already a strong ground swell in the US in favour of relaxing the NIH's guidelines. After all, nothing has yet happened to create an ecological disaster, has it? the disarmed hosts/vectors that we currently have, seem to be more than adequate; Nature may even have tried many of the scientists' new tricks without the benefit of guidelines. Well, the pendulum was bound to begin the reverse swing somewhere, soon. I hope that a sensible mid-position can be found, and held.

This book sounds the occasional wrong note but it tells an interesting story of the US honestly and well, warts and all. An index would have been useful. The UK has only a passing reference (to GMAG) at the very end where the guidelines are damned with faint praise—“probably generated considerable scientific red tape”. Coloured, perhaps, by Rogers' rose-tinted spectacles? □

R. J. C. Harris is Director of the Microbiological Research Establishment at Porton Down, UK, and a current member of the Genetic Manipulation Advisory Group.



Paul Berg (left) and David Baltimore (right)



Photos: NAS (Berg) and Noëleen Chedd (Baltimore)

of “libraries” from shotgun experiments, since, in the present state of genetic knowledge, the dangers of accumulating unknown, as well as known and recognisable, dangers, are not insignificant. Very well, these dangers are only potential, not actual. The public is unlikely to be impressed by such an argument. Wisdom after the event cannot be entertained.

The book contains an outline of many problems, but the solutions to very few, despite the author's recourse to the (recorded) wisdom of the experts. American industry, for whom the NIH guidelines are politically inhibitory, was particularly worried about two aspects; first, the maximum 10-litre scale proposed and, second, the necessity to declare to other research groups any safer host-vector system

while reassuring to the psyche, is hardly the line of defence . . .”. Since Berg lists “careless mouth pipetting” as a cause of breakdown of physical containment apparently in a very high category, the training and discipline of those who are to work in such laboratories obviously leaves an enormous gap to be filled. “The ideal safeguard”, wrote Berg (according to Rogers), “is ingeniously designed biological containment that can prevent escape and propagation—even with slobos doing the work on open benches”.

Yet another of the problems facing NIH's Advisory Committee on Recombinant DNA Molecules is simple enough to define. Scientists can hope to understand, and reason with each other; but where will the public stand? It is not to be expected that



# Molecular politics for novices

Sydney Brenner

*The Ultimate Experiment: Man-Made Evolution.* By Nicholas Wade. Pp. 162. (Walker: New York, 1977.) \$8.95.

A VAST LITERATURE has accumulated in the few years since the issue of recombinant DNA research made its appearance. The documents in the case already fill several shelves, and there seems to be no end yet in sight. What began as a laboratory technique followed by some doubts expressed by scientists quickly generated a worldwide debate which spread over an enormous range of scientific and social questions. Molecular biologists were thrust into an arena very different from their laboratories and found themselves embroiled in discussions first with clinical microbiologists and safety engineers and then with lawyers, philosophers and politicians, trying to solve what in many cases were, and still are, insoluble problems. The debate was played out on the public stage, often with all the actors in full frontal nudity, so that what began as domestic drama soon took on, as Jim Watson put it, "the aspects of a black comedy".

Indeed, there were times when one came to believe that the real issue at stake was human rationality; and the new subject of "molecular politics" still demands a fine sense of the ludicrous.

Nicholas Wade has now written a book to help the ordinary reader through the tangled maze. He gives a simple account of the techniques used to join DNA molecules together, to introduce the beginner in molecular biology to the world of restriction enzymes, plasmids and shotgun experiments. Here too, one will find explained physical and biological containment. But more important than this technical background is that the novice in politics will get a clear idea of the development of the issue, from its beginnings at the Gordon Conference in 1973, when concerns about the research were first formally expressed, right up to the recent debate in the US on legislative means for regulating work on recombinant DNA. As readers of his articles in *Science* will know, Nicholas Wade attended most of the meetings that were held, and he is able to communicate something of the atmosphere of the debate, particularly as it has evolved in the US. There, as some of the foreign scientists who attended the Asilomar Conference discovered to their horror, the Press attend meetings organised by Federal agencies. At Asilomar, it became clear that their presence would ensure that not only the content but also the style of the argument would be relayed to the outside world. The sole public

objector to the Press at the meeting, when asked to explain his behaviour by an irate journalist, remarked that he "believed in the inalienable right of consenting adult scientists to make fools of themselves in private". That exchange with a man who represented a newspaper that had brought about the downfall of the President of the country together with the fact that two-thirds of the participants were American, led to the perception that the discussion of the issue would be strongly affected by the political and scientific conditions of the US. As the book clearly shows, time has proved this to be correct. The action is set firmly on the American stage and there are only passing references to what was going on in the UK and other countries. True, very little was happening and then only on a more subdued level; but there can be no doubt that the Ashby Report was an important element in helping the Asilomar Conference to decide what work should be done.

The book can only be comprehended in the context of American society. We must remember that the issue arose at a time when the Watergate episode had suspended trust in governmental institutions, when the radical university movement had lost its focus with the end of the Vietnam war, when the honeymoon between pure science and government had ended, and when, even in the US, resources had become limited. Only these crude facts and not abstract principles can explain such bizarre events as the alliance between the radicals in Cambridge, Massachusetts, and the Mayor of that town.

The reader will quickly find that the debate abounds with difficulties and paradoxes. Take the argument advocated by Sinsheimer and Chargaff that new artificial recombinants could generate an evolutionary catastrophe. This raises a deep scientific question which cannot be dismissed simply by Chargaff's reference to "the evolutionary wisdom of the past million years". We have to ask whether change in the biological world is limited by the extent of genetic variation or by selective pressure. It is this question which lies at the heart of the debate, and it cannot be avoided.

In our own time, an immense change has been wrought in the microbes associated with Man and animals. The enormous increase in antibiotic-resistant bacteria cannot be ascribed to the creation of novel recombinants out of nothing, but, as everybody knows, has been the direct outcome of the widespread and often indiscriminate use of antibiotics. The genetic elements conferring penicillin resistance must have existed before the discovery of penicillin; they were enriched because



Brenner (left), Richard Roblin (right) and David Baltimore (seated) at Asilomar

Photo: Andrew A. Stern for NAS



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## Appendix

A standardized nomenclature for restriction endonuclease fragments

# DNA INSERTION ELEMENTS, PLASMIDS, AND EPISOMES

## EDITED BY

**A. I. Bukhari**, Cold Spring Harbor Laboratory  
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Capturing the excitement of the discovery of this new class of genetic elements—DNA insertions—and detailing the extraordinarily varied facets of insertion phenomena, this book stands alone as the first complete treatment of these new pathways for the reassortment of genetic information and the evolution of chromosome structure in both higher and lower organisms.

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Appendices: The eleven appendices to this volume contain detailed information, including complete bibliographies: List of IS elements and their properties; sequences for IS2; plasmids found in *E. coli*, *Pseudomonas*, *S. aureus*, other gram-positive bacteria and plasmids constructed in vivo and in vitro (their molecular weights, number of copies per cell, etc.); the genomes of temperate phages (molecular weights, intracellular forms, integration sites, etc.); restriction endonucleases (a complete list with recognition sequences); physical (endonuclease cleavage) and genetic maps of temperate bacteriophages  $\lambda$ , P1, P2, P22,  $\phi$ 80, and Mu; methods for cultivation and use of Mu.

our technology produced an enormous increase in the concentration of this chemical in the world. Natural mechanisms of recombination ensured that these would spread, given the selective pressure exerted. Thus, it could be argued that mere existence is not enough, even survival is not enough; for any element to come to dominate the world there must also be a strong selective advantage and the means for its transmission. It is not widely realised that the Western region of the US is one of the world epicentres of plague, yet this is patently not a serious disease in that country. The reason is not that every plague bacillus in California is securely locked up in a P4 facility but simply that the environmental structure of a modern civilised society is incompatible with the selective propagation of that organism.

These and other issues are still with us, and the story is by no means ended. Yet, in looking back over the past three or four years something positive has been accomplished. The research work

has been allowed to continue, perhaps at a slower pace than many people hoped for; it is the results that are now beginning to emerge that will bring the greatest justification when the tumult has quieted and the dust has settled; and what will remain for the historians of the future will be the insights the technique will have given into the structure of genes in higher organisms. Whether or not any of the other benefits promised by some molecular biologists will have become realised is hard to say. When they appear, however, it will be society and not science that will be judged, because some of us will then want to know whether these benefits are to be used for the good of all humanity or merely to satisfy the selfish interests of some industrial societies. □

*Sydney Brenner is Head of the Division of Cell Biology at the MRC Laboratory of Molecular Biology in Cambridge, UK, and will become Director of that Laboratory in 1979.*

## Rational agriculture

J. G. W. Jones

*The Famine Business.* By C. Tudge. Pp. 141. (Faber and Faber: London; St Martin's: New York, 1977.) £3.95; \$8.95.

In writing about the future of world food supplies, authors are often tempted by one of two easy options: first, optimism that resources will be found to enable mankind to maintain the *status quo*, which in many respects is inequitable and dangerous for large groups of the World's people; or, second, total disaster which could only result in global famine and a massive reduction in population through starvation. Mr Tudge avoids both these extremes and charts a much more difficult course for food production.

He introduces his subject by assailing the myths of population, energy use, and aid to developing countries; there can be no repetition of the economic growth experienced by the Western World since the Industrial Revolution nor a solution to the problems of feeding the developing nations based on technologically advanced agriculture. On the one hand he rejects the idea of the "world farm", where each area of the world specialises in growing what it is best able to, and on the other the idea of national self-sufficiency. The solution he proposes is one of "rational

agriculture" which, considering the difficulties, he defines remarkably precisely as "one that makes best use of the land, while meeting the nation's nutritional needs and gastronomic aspirations." The physical organisation proposed consists of (a) regional specialisation to a degree for beans, cereals and potatoes, and (b) local self-sufficiency for fruit and vegetables together with pigs and poultry fed on plate wastes. Establishing a rational agriculture would be impossible in capitalist systems but what kind of political and economic framework would be best is not stated. Capitalism is excluded on the grounds of its need for continuously expanding economies which are not sustainable in relation to food supplies unless irrational buffers, such as meat production from cereals or food processing, are introduced.

In discussing the nutritional implications of rational agriculture, the author asserts that man has always been omnivorous; any imbalance towards carnivorousness or herbivorousness is due to the omnivorous man's capacity to adapt to circumstances, and especially to the commercial promotion of meat, since the Industrial Revolution. The protein myth is predictably debunked. A plea is made to use the peasant cuisines of the World which were based on austerity and evolved to make palatable and interesting dishes.

The food processing industry is attacked as being concerned with unnecessary operations designed "to impose industrial techniques on food production". Many of its claims are quite contrary to the facts; variety,

seasonality and localisation of food supplies are all lost. Is "convenience" really achieved when time and energy is spent on distant marketing at hypermarkets rather than in the kitchen cooking? Particularly adverse comment is levelled at what Tudge calls "ersatz" foods. The price of meat analogues should be compared with the price of the beans from which they are made rather than with the price of meat; it would then be seen that not only are they inefficient in the use of resources but also uneconomic from the consumer's point of view. The industrial production of single-celled proteins is technologically too advanced for the Third World, and when all is said and done is only suitable for animal feed and thus inefficient.

The book cannot be described as cranky. It is logical and engaging to read, and, except in his attacks on the food processing industry, the author is commendably restrained in his consideration of the implications of rational agriculture for technology and for capitalism. Politics, nutrition and commerce have been mixed with iconoclasm to produce a book which should appeal to the idealist in every reader.

*J. G. W. Jones is Senior Lecturer in the Department of Agriculture and Horticulture of the University of Reading, UK.*

## Curriculum change and social climate

Mary Waring

*Science Textbook Controversies and the Politics of Equal Time.* By D. Nelkin. Pp. xi+174. (MIT Press: London, Cambridge and New York, 1977). £9.10; \$12.95.

In 1969, the Californian State Board of Education's guidelines for school biology ruled that creation theory, as given in Genesis, be taught as a viable, scientific alternative to the theory of evolution, equal time being accorded to both. The target was, clearly, National Science Foundation-funded BSCS texts, with their evolutionary underpinning, and the ruling represented a victory for local creationist pressure groups. Attacks on evolution were nothing new, and biologists responded only slowly; eventually, however, legal and political strategies seemed to be restoring the situation. Then, a nationally-linked network of creationist activists turned their attention to a NSF-funded social science



course, *Man: A Course of Study* (MACOS), whose evolutionary underpinning was far more explicit in its use of animal behaviour to develop concepts about the nature of man, and which fostered ideas of cultural relativism. The course had already received wide acclaim and, by 1974, was being used in some 17,000 schools in 47 states. In 1975, however, after several local controversies, and following Congressional airing of the issue, funds for MACOS were terminated, sales plummeted, and a major re-evaluation of the NCF's (federal) funding of science curricula was instituted.

The causes and effects of action can never be explained in their own terms, but only in relation to all the events—and the network of interests, vested or otherwise—of which the action is part. Clearly, creationists were merely catalysing processes already latent in American society, and Professor Nelkin therefore sought for more widely-dispersed social and political tensions. Her interest had been aroused initially by the fact that creationists were representing themselves as *scientists*, a claim that enabled them, in attacking science textbooks, to circumvent dismissal on grounds of irrelevance or of infringement of academic freedom. Moreover, their focus on the highly sensitive area of the role of schools in value transmission enabled them to capitalise on a seemingly widespread concern over the erosion of traditional moral and religious values. Other significant tensions were concern for the control of hitherto 'unaccountable executive bureaucracies', for a questioning of scientific 'authority', and for lay participation in decision-making.

Professor Nelkin's account makes fascinating reading and her analysis is valuable in drawing attention to the significance of social climate in educational decision-making, but it is not entirely satisfactory. This is, perhaps, partly because boundaries have to be drawn somewhere (the area is a very complex one), but it is also because there is some woolliness in the discussion of, for instance, the nature of science and, in particular, the relationships between the sciences (especially when social sciences are included), and of the standing of evolutionary theory. The fact that MACOS is in fact intentionally directed towards the development of judgement and tolerance in a pluralistic, democratically orientated society is not made clear in her concern to juxtapose scientific and social values, and we get no explanation for social scientists' failure to defend their project. (Biologists do not seem to have regarded it as their concern.) Curiously, too, no mention is made of the possible influence of economic recession on both sales and funding.

Nevertheless, there is a mine of information and careful documentation here. The story is well told and is absorbingly interesting. The book is a most useful addition to the literature on curriculum change and social

climate and on pressure groups in science education. □

Mary Waring is Lecturer in Education (Social Biology) at the Centre for Science Education, Chelsea College, University of London, UK.

## Policy research perspectives

Stuart S. Blume

*Science, Technology and Society: A Cross-Disciplinary Perspective*. Edited by Ina Spiegel-Rösing and Derek de Solla Price. Pp. 607. (International Council for Science Policy Studies: London and Beverley Hills, California, 1977.) £20.

THIS volume offers a 'state of the art' review of that somewhat diffuse research field called here Science, Technology and Society (STS) studies, directed "mainly toward scholars in the various constituent sub-fields" (p3). Because the structure and value of the book can only be understood, and its strengths and weaknesses assessed, in the light of some appreciation of the structure of the field, I must begin with some general remarks on the study of scientific activity.

Research which the reviewer may choose to subsume under a heading such as STS in order to explore or (as intended here) to stimulate the 'intellectual integration' of the field is actually carried out from a variety of perspectives. Thus, sociology of science and the economics of research and development are both central, but most people working in these areas owe their principal allegiance to, and derive their research perspectives from, the well-established disciplines of sociology and economics. At the same time, there has emerged in the past 10–15 years a substantial multidisciplinary research activity, to which the existence of (for example) the Science Policy Research Unit at Sussex University, the Science Studies Unit at Edinburgh, and the Society for Social Studies of Science all bear witness. The fact is that the rapid growth, and institutionalisation, of both disciplinary and multidisciplinary research has not resulted in any real crystallisation of the field.

On the face of it, this apparently transitional intellectual structure has many parallels. Many new fields of scientific or technological research pass through phases in which some new integrative perspective competes, intellectually and professionally, with its parent disciplines. The early days of, for example, molecular biology, oceanography, radio astronomy, and materials science were like that. STS in fact differs from these in that 'the' new multidisciplinary perspective, which

ought to provide the basis for a new crystallisation, is itself fragmented. Broadly speaking, one nucleus has been science policy: the practical problems of allocating resources to science, organising research, stimulating and evaluating technological innovation, and so on. (Salomon's chapter in the volume under review shows how science policy research has been, in part, a response to the development and politicisation, of national science policies.) Equally broadly, a second nucleus has derived from the growing conviction among (some) historians, philosophers, and sociologists of science that the production of scientific knowledge has to be seen as having both epistemological and social dimensions which in fact interact. Thomas Kuhn's famous book *The Structure of Scientific Revolutions* was a major stimulus to this recognition of the need for cooperation.

What we have today, therefore, is a considerable variety of research approaches, and related problems and priorities, some of which (and these far from dominant) imply the need for greater cooperation between two or three relevant disciplines. For example, in addition to a perspective which relates sociologists, philosophers and historians of science, another begins to link economists and political scientists in the study of technological change.

In grouping their contributions into three sections, Ina Spiegel-Rösing and Derek Price have made a similar kind of categorisation of orientations to the field. The first section, seeking to be integrative, focuses mainly on the origins, institutional structure and cognitive structure of STS as 'a' subject. The second section looks at the achievements of social studies of science, taking the relevant disciplines *seriatim*, whereas the final one considers science policy-related studies, and is mainly written by political scientists. Because part 1 is closely bound up with the scope, utility, and future of the subject as a whole I will postpone discussion of it for the moment.

Part 2 contains chapters by a sociologist, two historians (of science and of technology), an economist, a psychologist, and a philosopher. One turns to these chapters with numerous hopes: of an outline of the various perspectives current in each discipline, their achievements, and their potentialities (especially for greater cooperation with other disciplines); and of some communication of the excitement of the research and of its currently crucial problems. Although some contributors (notably Fisch on

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psychology of science) are greatly handicapped by lack of progress to report, on the whole one is not disappointed.

MacLeod's chapter on the social history of science is particularly successful. He shows that this remains a minority perspective within the history of science, because it rejects the common assumption that scientific developments can be understood in terms purely of their intellectual antecedents, without reference to the environment of discovery. Moreover, for the social historian (though not for the orthodox 'internalist'), this environment is itself a subject of interest, providing potential linkages with other disciplinary perspectives. One turns the final page of MacLeod's chapter both informed and enthused.

Freeman (on the economics of R&D) and Mulkay (on the sociology of science) provide the highly professional treatments one expected. Freeman outlines the perspective on science and technology found in each major school of economic thought, from Adam Smith, and indicates the findings of recent empirical work on technological change. He devotes considerable space to the kinds of question which might lead to greater cooperation between economists and other specialists (for example, whether government investment in industrially orientated R&D should supplement or complement—in terms of industrial sector and timescale of benefits—what firms themselves do), and ends with a plea for a "political economy" of R&D.

Mulkay concisely summarises the major achievements of the sociology of science since R. K. Merton's pioneering work of the 1930s. He might only be faulted for saying too little about the frontier points at which sociologists are coming to work with philosophers on the one hand (partly treated by Böhme) and political scientists on the other.

There is considerable overlap in part 3 between Sapolsky (on science, technology and military policy), Schroeder-Gudehus (on foreign policy) and Skolnikoff (on the international system), although each writer offers valuable insights. I did feel the need, however, for some (even implicit) notion of the uniqueness and coherence of science and technology; more sociological understanding might have been helpful. An adequate understanding of the changing relationship between scientists and political power (treated by Lakoff) must involve appreciation of the effects of this relationship on the ethical and normative system of science, the constraints on the occupants of advisory roles, and so on. These are (partly) sociological questions.

Still more important, though seemingly unrecognised, is the need for some kind of theory of technological change. For example, Sapolsky makes the interesting observation (p458) that the process of weapons development may have become

'autonomous': "so oriented to serving its own internal needs that it weakens security by generating weapons which are too costly to produce" in sufficient quantities. Layton, writing about the history of technology, refers (p127) to demands for more socially responsive technology as requiring "a change in the inner structure of technology". But what is this "inner structure" and how does it change?

In Nelkin's chapter, we turn from the disciplines of the social sciences to the real world problems posed for policy-makers and citizens by modern science and technology. How can we make social assessments of technology? How can adversary (for example, court) proceedings cope with technical questions? When should we mount a giant "Manhattan Project" attack (for example, on cancer) and when should we more wisely invest principally in basic research? STS research has few answers here, and this has to be recognised in assessing the current usefulness of the field, and the book, to those who are concerned non-specialists.

I now return to part 1, which is most relevant to a general overview. It is certainly recognised here that the current state of STS research is not such as to throw much light on practical questions (other than in some very narrow areas). But part 1 should have gone further. These practical questions surely require,

as most practical questions do, an integration of insights of a multidisciplinary nature. It is clear from the book that in this field such integration, such crystallisation, has made little progress. And yet in many of the contributions one finds reference to problems and perspectives that promise or imply cooperation with other disciplines. The locus of these cooperative possibilities, which could have been traced out, might have served as a beacon to those wandering this terrain.

Some of those who work on STS will take pride in the achievements charted in this book. Others, among whom I include myself, are perturbed rather that so much has happened in so piecemeal, so incoherent a fashion. The vast creative effort which might have forged of this book a motor of real progress has not been made. Perhaps no-one could have done it, and the criticism is unfair. I can certainly say that anyone deriving sociological, economic, philosophical or policy research problems from science and technology should read the book. I cannot really recommend it more widely, and indeed I rather hope that my erstwhile science policy colleagues in Whitehall do *not* read it. It will not persuade them of the usefulness of this kind of work!

*Stuart S. Blume now works in the Department of Sociology and Psychology at Chelsea College, University of London, UK.*

## Utopian dream

Marie Jahoda

*Civilization in Crisis: Human Prospects in a Changing World.* Pp. 303. By J. A. Camilleri. (Cambridge University: Cambridge, New York and London, 1977.) £7.50 (paperback £2.50).

CAMILLERI, a young Canadian political scientist at Le Tobe university, has performed a *tour de force* in this book. He aims at nothing less than a comprehensive diagnosis of the many factors which account for the "decadence of industrial culture", to establish a "utopian vision" and to propose a "realistic strategy for change".

The major part of the book is devoted to the diagnosis of the current crisis which Camilleri regards as a crisis threatening the survival of man as a species. His arguments go, however, in scope and spirit, beyond the usual doomsday forecasts, even though he uses ideas and assertions from that source where they fit his purpose.

The major symptom of the crisis is, according to Camilleri, widespread alienation: "... political apathy, social anomie and mental or emotional dis-

order are the product of the dual process of domination and alienation to which the technological society subjects the human psyche" (p13). Drawing on a wide range of literature, including the various social sciences, philosophy, cultural critiques and analyses of current economic, political and military events on a world-wide basis, he suggests that only a radical change in international and national institutions can avoid disaster.

In discussing the economic and political dilemmas of the world and their manifestations in increased inequality, underdevelopment and instability technology emerges as the major villain, leading to bureaucratic centralisation in capitalist and advanced communist societies, testing existing institutions beyond their limits and creating an intolerable imbalance. But on p222 Camilleri pulls himself and the reader sharply up in recognising that he is in danger of throwing the baby out with the bathwater: "... we may have created the erroneous impression that technology lies at the root of the modern human predicament. The problem of domination and dehumanisation that we have been describing cannot be attributed to technology as such, but only to the specific role assigned to it by the ideology of industrialisation." But he remains evasive on

how international inequalities can be reduced without some ideology of industrialisation in poor countries. There are various hints in the book suggesting that the Chinese experiment in social organisation may be one way out of the dilemma. But Camilleri is too concerned with the liberation of the individual to suggest a wholesale transfer of an experiment whose authoritarian character the Western world is beginning to realise as the perhaps inevitable counterpart of its remarkable virtues and achievements.

Camilleri's "utopian vision" is culled from a number of thinkers of most diverse outlook—Marcuse and Pope Paul VI, Marx and Teilhard de Chardin, Mao and Gestalt therapists, Illitch and (surprisingly and only in a note) Skinner. The author is right, of course, in saying that any critique of the present involves at least logically a concept of what could be, and equally right in not proposing a blueprint but rather a set of human values which should be maximised in a new cultural synthesis. Central to this deliberately vague utopia is the small organic community where "the liberation of human sensitivity and sensibility is made a primary goal of social interaction" (p187).

It is to the author's credit that he does not shirk the question of how to make this often dreamt dream come true, even if his answer is more appealing for its sincerity and morality than convincing as a solution. Camilleri advocates a non-violent cultural revolution which he sees already emerging in the rich world: "Once the counter-institutions, whether it be free universities, underground churches, free presses, community schools, worker-directed enterprises or neighbourhood-controlled welfare services, have demonstrated their efficiency and legitimacy through a continuous process of innovation and experimentation based on service and direct participation, the established institutions are likely to collapse with little or no coercion required..." (p254).

One can hardly blame the author for not having solved all the world's problems. Nobody else has done so either. But such naiveté, together with many unexamined assertions and some inconsistencies in the book, overshadow its positive features.

The value of the book to a reader wishing to appraise the evidence on which the author bases his arguments would have been enhanced, had the index included references to the Notes.

Marie Jahoda, Professor Emeritus of Social Psychology, is now senior research consultant to the forecasting programme of the Science Policy Research Unit at the University of Sussex, UK.

## Hard and soft paths

P. F. Chapman

*Soft Energy Paths: Towards a Durable Peace.* By Amory B. Lovins. Pp. 254. (Ballinger: New York; Wiley: Chichester, UK, 1977.) Hardback \$15.40; £9 Pp. xx+231. (Penguin Books: Harmondsworth, UK, 1977.) Paperback 95 pence.

An energy policy involves a projection of energy demand at some future date coupled to a means of supplying the requisite quantities of energy in appropriate forms. Although there are radically different policies advocated, each with its own mix of supply technologies and demand estimates, virtually all policy analysts are agreed on the ground rules that must be used to evaluate or judge a policy. The first rule is that the demand projection and methods of energy supply should neither require nor impose any substantive change in consumers' lifestyle. The second is that all the technologies involved, either in supply or utilisation, should either be proven or have a demonstrably high chance of feasibility. The third rule is that the policy should provide energy at least cost. If this latter cannot be demonstrated then it is essential to demonstrate that all investments show an adequate return on capital. The differences between advocates of different policies can, under these agreed rules, be boiled down to different choices of important parameters, with each author accusing others of making choices of convenience. Since many of the important parameters are in principle unknowable, this leaves plenty of room for endless, and fruitless, debate.

In his new book, *Soft Energy Paths*, Amory Lovins attempts to use the agreed rule book to show that the "hard technologies" advocated by the establishment are significantly less desirable than the "soft technologies" proposed by environmentalists, conservationists, lovers of peace, and... Amory Lovins. The basic thesis of the book was published by Lovins in *Foreign Affairs*, an influential US quarterly. The article is reproduced as chapter 2 of the book, the remaining chapters serving to embellish and document the arguments. Although the approach and data in the book are clearly aimed at influencing US energy policy, Lovins makes it clear that the same principles and analyses could, and should, be applied in all industrial nations.

The 'hard' path described in the book is based on the policies of a number of US agencies, including the Energy Research and Development Agency. This policy has energy demand (in primary energy terms) doubling by the end of the century and increasing by as much again by 2025. Over the 50-yr period, oil and gas decline from a 75% market share to about 10% with coal and nuclear power accounting for both the growth and the substitution. Lovins argues against this policy in terms of changes in lifestyle and unacceptably high costs. He shows that the massive use of technical resources, the potentially enormous environmental damage and the security measures required for a massive nuclear programme, will alter the way of life of most Americans. He also argues, with considerable conviction, that the development of nuclear power will lead to the widespread proliferation of nuclear weapons with potentially enormous effects on 'lifestyles'.

Lovins' critique of the hard path on cost grounds is similar to many previous analyses. There is no doubt that the costs of nuclear systems are becoming embarrassingly large, and there seems to be no decrease in their escalation at a rate substantially faster than the rate of inflation. To this embarrassingly high cost, Lovins adds the equally enormous costs of distributing electricity to consumers. He then makes the numbers absurdly high by dividing by an unrealistically low 'load-factor'. This is silly, since it provides ammunition for those who would knock down an otherwise sound and important argument.

Underneath the critique of the hard path, there are a number of principles which show Lovins' previous training in physics. The first is an obvious distaste for waste of a physical commodity which, by any route, is going to be more expensive in the future. The second is the mismatch implied between the high quality of energy supplied to consumers (electricity) and their low quality energy requirements (low temperature heat). Lovins attacks the question of mismatch, directly as a matter of principle, without drawing on the obvious economic argument that rising prices will push consumers towards more appropriate fuels. This is perhaps because Lovins subscribes to Galbraith's thesis which puts sovereignty in the hands of producers not consumers.

Improving the match between energy supply and end-use function is one of the major starting points for the 'soft' path synthesis. Another is that if one can use ambient energy sources, particularly wind and solar, then one

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can avoid most of the costs of energy transmission. To these significant advantages, Lovins then adds a number of very dubious cost advantages associated with small scale mass production (as opposed to large scale one-offs). It is here that Lovins falls into the ubiquitous trap of choosing data that happily proves his case. Two examples illustrate the type of problems involved.

First, Lovins suggests that car engines provide a system of electricity generation at a cost of \$40 per kW compared with power station costs of more than \$400 per kW. Lovins omits, however, to cost the 100 kW generator that needs to be coupled to the car engine. More seriously, his argument for mass production is undermined when it is realised that a car engine is worn out after 4,000 h of running time, whereas a large generator is expected to run for 6,000 h per yr for 20 yr or more.

The second example concerns the cost of solar energy systems. Lovins is only concerned with the cost of active solar systems, employing flat plate collectors and large hot-water heat stores. He arrives at a figure of \$100/m<sup>2</sup> of collector *plus* a m<sup>3</sup> of storage. In the UK, an optimistic cost for solar collectors is £50/m<sup>2</sup>. The cost of storage in the UK has been put at £300/m<sup>3</sup> by the Buildings Research Establishment. By using cheap agricultural tanks and the cheapest methods of installation and insulation this cost can be reduced to £40/m<sup>3</sup> for moderate tank sizes (over 40 m<sup>3</sup>). Thus UK costs are at best £90 per (m<sup>2</sup>+m<sup>3</sup>), equivalent to £150. This estimate is, however, still too low, since it ignores the cost of loss of house space and control equipment. The 50% difference between the most optimistic UK figure and Lovins' data is sufficient to cast serious doubts on the cost-effectiveness of active solar systems.

There are other serious criticism of the proposed 'soft path'. Throughout the book Lovins fails to produce comprehensive data on how much energy is to be supplied by what source for use by which consumers. Many attractive back-of-the-envelope calculations fall down when examined in detail, especially in complex energy systems. There is also no adequate discussion of the environmental effects of the soft path. In this respect, windmills are clearly cast in doubt, and outside the US there are few countries who could afford much acreage for growing 'biomass' fuels.

Although Lovins' book does fail on a number of technical issues, this is more than compensated by his breadth of approach. Energy policy questions are cast in a very general sociopolitical framework and a number of important questions raised. Of these, the relation-

ship between nuclear power, centralised control and weapons proliferation are the most important and urgent. Is any energy policy important enough to threaten every nation's foreign policy?

Although Lovins clearly identifies many of the central issues, one is left with a number of important questions unanswered. The last four years have seen some remarkable shifts in attitudes towards the alternative (or soft) energy technologies with most nations now actively supporting a very diverse energy research and development programme. There remains, however, an enormous commitment to nuclear power. The costs are embarrassing both the utilities in the US and the Central Electricity Generating Board (CEGB) in the UK. (Here, it is interesting to note that the CEGB is supporting British Nuclear Fuels Limited at Windscale, despite their admission that reprocessing will cost them about £300 million more than obtaining fuel from uranium ore).

## Energy forecasting

Walt Patterson

*Energy or Extinction? The Case for Nuclear Energy.* By Fred Hoyle. Pp. vii+81. (Heinemann Educational: London, 1977.) Paperback £1.50.

COSMOLOGY does not, on the face of it, have much to do with the subtleties of terrestrial economics and politics. Nevertheless, Sir Fred Hoyle has taken a flying leap out of the galactic depths and into the middle of one of today's most immediate and heated controversies. Sir Fred, of course, is no stranger to controversy. He is, however, something of a stranger to energy policy, as he demonstrates repeatedly in his lively polemic *Energy or Extinction?* He writes, as usual, with flair and enthusiasm. All too often, however, he finds himself out of his depth.

The tone is set by Sir Alan Cottrell's Foreword to the book: "It is about energy: about the alarming prospect that oil will soon run out and not be replaced by anything else. It shows that—contrary to an influential belief—we do *not* have time, that there is *no* practical alternative to nuclear energy . . ." After this outburst, Sir Alan goes on to deplore the "hysteria of the anti-nuclear environmentalists". Both the Foreword and the book it introduces bear witness that environmentalists have no monopoly on hysteria. Sir Alan's allusion to the book's "refreshing commonsense" runs afoul of its

Wherein lies the source of this commitment? Is it the massive investment in nuclear technology? Is it the enormous institutions built-up on nuclear technology employing so many highly qualified scientists and technologists? Or is it perhaps the military link? Lovins book is causing ripples in the US and may encourage President Carter to go further with his non-proliferation policies. It will certainly provoke interest and controversy in the UK. But before any action can be taken on a 'Soft Energy Path; Toward a Durable Peace', someone will need to analyse the soft path in far greater detail and be able to account for the commitment to the hard path policies presently being followed by all the industrial nations of the world. □

*P. F. Chapman is Senior Lecturer in Physics and Director of the Energy Research Group at the Open University, Milton Keynes, UK.*

very title; do we really have to choose now between "energy or extinction?" It is an emotional catchphrase that begs almost all the important questions.

Sir Fred's approach to energy policy is based on a series of non-negotiable assertions couched in stark all-or-nothing terms: "There can be no disagreement with the statement that world reserves of coal, oil and gas can provide an adequate energy source for only a limited future . . . nor can it be contested that most of the world's population . . . will die in a disastrous catastrophe should an adequate energy source not have been developed by the time that reserves of coal, oil and gas become exhausted . . . nor can there be any serious debate over the statement that the only alternative energy source *presently known to be technically viable* (italics in original) is energy from the nuclear fission of uranium or thorium". If Sir Fred genuinely believes that none of these assertions can be disputed, he has somehow failed to notice the boiling ferment now engulfing energy policy worldwide.

He has also overlooked a fundamental consideration which is likewise absent at key places elsewhere in the book: the matter of cost. Even "reserves" of fuel depend critically on the extraction cost assumed, as well as the value assigned to the fuel in use. "Most of the world's population", however, presently depends largely on forms of energy which are not bought and sold. Technical "viability"—surely a contradiction in terms—is long since proven for many energy technologies, and is not the central problem. The primary problems are economic—how much money and time we are prepared to

spend on various aspects, especially investment; social—the costs and benefits, and how they are to be apportioned, especially employment; and political—who shall decide, and how. Despite Sir Fred's suspicions, the factor now impeding nuclear development is not the third but the first of these: the cost and the lead-time.

Sir Fred belongs to the traditional school of energy forecasters, whose motto is "Think of a number and double it". He notes that Britain's per capita annual use of energy is only about half that of the US: "this of course is the over-riding reason why the standard of living is lower in Britain than it is in America. By doubling the British energy flow, our standard of living would rise inevitably towards the American level". That being so, why do we not simply turn up the central heating and open all the windows? The putative link between GNP and energy use looks more tenuous every year, to say nothing of that between GNP and 'standard of living' in meaningful terms. Sir Fred, however, desires "to raise the standard of living of everybody in the world to the American level"—presumably by American cultural standards, universally acknowledged as the acme of human accomplishment. It seems not to trouble him that the immediate requirement of most of the planet's people is for local energy systems, using local skills and most of the planet's people is for local money. In most Third World countries, capital-intensive centralised electricity systems are a comprehensively inappropriate misallocation of effort, as even the World Bank is coming to accept.

Sir Fred is convinced, however, that this is how we must deploy our resources. He is concerned with energy supplies 30,000 years hence, but has a shakier grasp of more relevant time-scales. "It is a mistake to think that conservation could go very far towards mitigating our future need for energy. Conservation could moderate the need, but by no great margin, for *if the margin were great, conservation would have happened already*". Our present pointlessly wasteful infrastructure—buildings, transport, industrial plant—was established during a period of absurdly cheap energy. This infrastructure cannot be replaced overnight with one more appropriate to a time of high energy price. Such replacement, however, is already underway; and it does not in any way imply—in Sir Fred's phrase—a "hairshirt economy" (see, for instance, the evidence given by Friends of the Earth to the Windscale inquiry).

Sir Fred's lack of first-hand acquaintance with the work and views of Friends of the Earth led him and his

publisher into a slight contretemps. The opening chapter of the book suggests in lurid terms that critics of nuclear power like Friends of the Earth are agents or dupes of the Kremlin. Before the book's publication, however, Sir Fred and Heinemann added and circulated a statement, withdrawing all imputations of 'reds under the reactors'; and two staff members of Friends of the Earth were guests at the book's publication party.

There are a few lapses which Sir Fred should have caught: "molecules" of salt? His discussion of the nuclear option itself suggests that he needs further homework here as well: "fuel elements" or "fuel assemblies", but not "fuel cells", and power reactor control rods are not made of cadmium. His discourse on radioactive waste commences with another brisk wave of the hand: "I have always been asked about the 'problem' of the disposal of radioactive wastes. There is no problem, and the thought that there is, if the concern is genuine, can only come from ignorance and from a fear of the unknown". Perhaps he should have a word with Sir Brian Flowers, whose ignorance and fear of the unknown are conspicuous by their absence. Sir Fred's otherwise generally lucid prose ties

itself in knots during his attempts to banish the non-problem. He also gets the half-lives of both strontium 90 and caesium 137 wrong.

Much the most unsatisfactory paragraph in the book, however, is his extraordinary dismissal of the issue of nuclear weapons proliferation. Rarely have I seen such wilful sophistry as the assertion that linking nuclear energy and nuclear bombs is on a par with linking "eating a piece of chocolate and exploding a hand grenade" because "both (are) manifestations of chemical energy". This will not do, and Sir Fred should know better.

It is ironic that—assuming we move quickly to resolve the nuclear weapons problem—the so-called "anti-nuclear environmentalists" are today the optimists, who foresee an abundance of options and are working eagerly to respond to the challenge. Those who insist that the choice for humanity now lies between nuclear energy and freezing in the dark are—in Sir Fred's verbal boomeranging—"scaremongers whose prime concern is to frighten people". Cheer up, Sir Fred—from where we sit the view is really rather encouraging. □

Walt Patterson is Energy Consultant to Friends of the Earth (London) Ltd.

## Epic work on climate

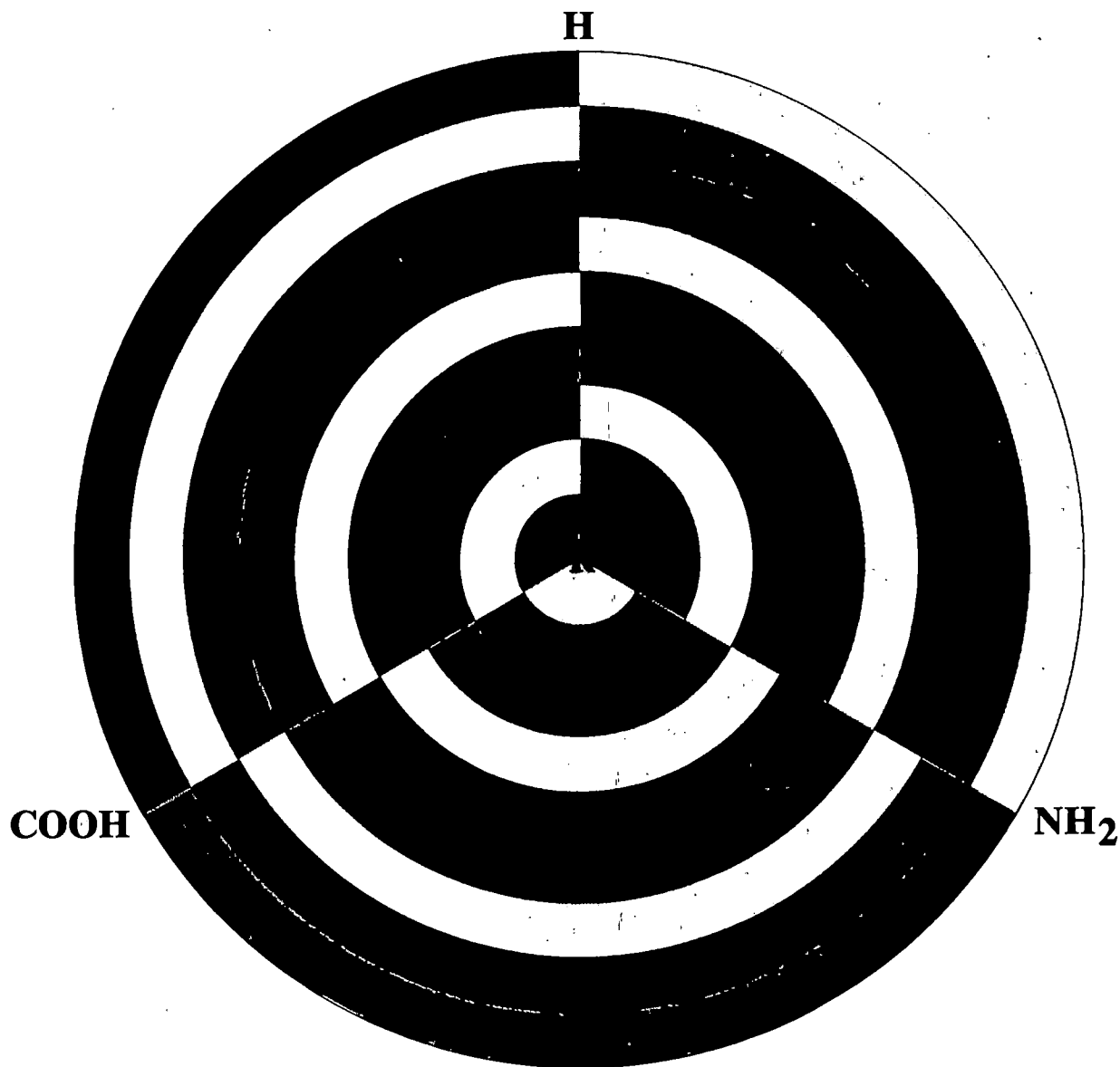
John Gribbin

*Climate: Present, Past and Future*. Vol. 2: Climatic History and the Future. By H. H. Lamb. Pp. 835. (Methuen: London; Barnes and Noble: New York, 1977.) £38; \$85.

THE arrival of the second volume of Professor Lamb's epic on climate has been eagerly awaited by everyone working on studies of the climate. The first duty of any reviewer is to report that the book is no disappointment, fulfilling the high expectations of those who have already found the first volume indispensable. That said, it might seem that further comment is superfluous; but since 1972, when volume 1 of *Climate: Present, Past and Future* appeared, debate about climatic change has moved so much into the realms of the planners, politicians and concerned laymen with no specialist training in the subject, that the book is now of far wider significance, and likely to have a much broader impact, than even the author could have imagined when he began his mammoth task twelve years ago.

Recent vagaries of the Earth's atmospheric system have been front-page news around the world, and include record-breaking drought followed by record-breaking precipitation in England; simultaneous severe drought in the western US and heavy snowfall in the east; failures of the monsoon in some parts of the world; and frosts in Brazil, which have been used as an explanation of recent dramatic increases in the price of coffee. The latest annual report of the UK Meteorological Office was the subject of a first leader in *The Times* in which the writer commented "there seems little doubt that the world's climate has been changing", and was taken to task by a member of the School of Geography in Oxford who argued that it has all happened before and refused to accept "that recent weather events in Britain provide reliable evidence for a significant climatic change".

When the world's weather seems to be turned topsy-turvy, but specialists can be found prepared to argue any of the cases that: (1) nothing unusual is happening; (2) we are hastening towards a new Ice Age; or (3) present troubles are caused by the greenhouse effect of human activities which produce carbon dioxide and warm the globe, the wretched non-specialist—



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**Karl R. Popper**  
Penn, Great Britain  
**John C. Eccles**  
Contra, Switzerland

# The Self and Its Brain

66 figures, approx. 10 tables. Approx. 610 pages. 1977

Cloth DM 39,—; US \$ 17.20

ISBN 3-540-08307-3

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## Contents:

Materialism Transcends Itself. The Worlds 1,2 and 3. Materialism Criticized. Some Remarks on the Self. Historical Comments on the Mind-Body Problem. Summary.—The Cerebral Cortex. Conscious Perception. Voluntary Movement. The Language Centres of the Human Brain. Global Lesions of the Human Cerebrum. Circumscribed Cerebral Lesions.—The Self-Conscious Mind and the Brain. Conscious Memory: The Cerebral Processes Concerned in Storage and Retrieval.—Dialogues.

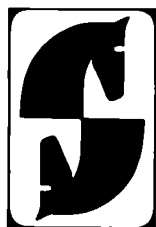
This book is timely, as it appears at a point of impasse between philosophy and science. It creates the first link between the philosophy of the self and neurobiology. In dealing with the self, philosophers have so far taken little account of scientific knowledge of the brain; scientists, for their part, have traditionally avoided philosophy in favour of purely material evidence.

Eccles (a neurobiologist) and Popper (a philosopher), both believers in dualism and interactionism, consider the existence of consciousness one of the greatest riddles of cosmology.

In Part I, Popper discusses the philosophical issue between dualist or even pluralist interactionism on the one side, and materialism and parallelism on the other. There is also a historical review of these issues.

In Part II, Eccles examines the mind from the neurological standpoint: the structure of the brain and its functional performance under normal as well as abnormal circumstances, for example when lesions (especially those surgically induced) are present. The result is a radical and intriguing hypothesis on the interaction between mental events and detailed neurological occurrences in the cerebral cortex.

Part III, based on twelve recorded conversations, reflects the exciting exchange between the authors as they attempt to come to terms with their conflicting opinions. This part preserves the intimate quality of these dialogues, and shows how some of the authors' viewpoints changed in the course of these daily discussions.



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who, in this case, may be involved in attempts to improve agricultural productivity to meet the needs of a growing global population—needs some secure foundation on which to base his interpretation of climatic events. Professor Lamb's books provide that secure foundation, and the second volume is of particular importance to anyone concerned in an empirical way with changes in climate, concentrating as it does on climatic history and on implications for the future.

The simplest and most reliable guide to the climate is that if something has happened before it can happen again. Professor Lamb's look at what has happened before covers successively, and in increasing detail, the long history of the Earth, the Quaternary, postglacial times, the historical record, and the period of instrumental records. This natural division of attention reflects, of course, the amount of detailed information available, but also concentrates on the periods of most interest now—the most recent fluctuations in climate. With an outline of more distant, long term events as background, the changes since the most recent full glaciation provide the appropriate insight into what may be happening to climate today. Suggestions that man's activities are solely responsible for increased Saharan desertification, for example, should be considered in the light of evidence about past development of the desert; this would seem to indicate that both the Sahara and Kalahari deserts spread towards the equator during periods of cooling climate.

Cooling phases are likely to be of particular interest at the present time, since the consensus of various forecasts assembled by Professor Lamb is that we are likely to see a continued global cooling over the next 30 years or so. This is not to say that we yet have reliable means to forecast such changes in detail, but "encourages belief in the possibility of developing a real forecasting skill in relation to such periods ahead, and suggests that some useful guidance has already been obtained from this scientific research". The joker in the pack, however, is the prospect that mankind's activities, most likely the carbon dioxide greenhouse effect, will introduce a significant new variable into the climatic system just at a time when we are on the brink of understanding the natural processes.

Professor Lamb's great achievement is that he has assembled in one place a vast store of information about the climate, encapsulating present knowledge at this critical watershed in the development of such an understanding. As a sourcebook for everyone working on the subject, the book would be

invaluable even if it were not well written; in fact, the author presents his material (from the implications of plate tectonic theory to the effects of mankind's activities on climate) so clearly that the volume would be a pleasure even to the casual reader who might dip into the sections on historical changes in climate and effects on human activities. Alas, I use the word "might" advisedly; for the price of the present volume is so great as to preclude any casual reader, along with many who have a serious interest in the subject.

At £38 (in the UK) for a single volume—even one of major importance, well written and more than 800 pages long—the publishers must surely be reaching the point of diminishing returns. Can any working scientist possibly buy such a book? And, indeed, can all of the libraries that might wish to have it on their shelves justify the cost? It must also be mentioned, in view of the large capital investment such a book represents, that although the volume's presentation and layout is, overall, of a high standard many of the figures have not been re-drawn into a standard format, but are simply reproduced from their original sources. Although all are acceptably clear and no harm is done to the intelligibility of the book (except where the labelling of the figures is retained in its original Norwegian), this is hardly the kind of penny-pinching usually associated with such an investment. The presentation and cost of the two volumes may have seemed appropriate when the project was initiated, but in view of the much more widespread interest in the topic now it would be good to see the whole

work available in more accessible format—perhaps as a series of three or four paperbacks which might be purchased individually as finances permitted.

But for anyone fortunate enough to possess the book and its companion volume the intrinsic value is unquestionable. It is impossible to do justice to Professor Lamb's achievement in the short space available here, and it will take months to begin to absorb the impact of this appraisal of the background to present-day climatic problems—followed, I am sure, by a lifetime in which it will remain a much used and valued reference. Perhaps the final words should be left to the author. First, a statement of intent from the Introduction which aptly describes the work: "This book is an attempt to marshal existing knowledge of climate and its behaviour, so as to provide a basis on which forecasting techniques will have to be developed".

And, finally, from a footnote (the many comprehensive footnotes are themselves one of the major delights of the book) commenting on those historians who do not accept that climatic change was contributory to the cultural decline and other disturbances of the Middle Ages in Europe, a remark which is strikingly apposite in view of the current climatic debate: "There is great need for open minds and a careful integration of knowledge". □

*John Gribbin is a Visiting Fellow at the Science Policy Research Unit, University of Sussex, UK.*

## Encyclopaedic jungle

Peter J. Smith

*Planet Earth: An Encyclopaedia of Geology.* Edited by Anthony Hallam. Pp. 319. (Elsevier/Phaidon: Oxford, 1977.) £7.95.

THE publishing world seems to have gone mad as far as the production of geological dictionaries, encyclopaedias and the like is concerned. I reviewed three such volumes earlier this year (*Nature*, 266, 101; 1977); several were then already in existence; another appeared a month or so ago; I have a new one to review here; and the publication of at least two more is imminent. This field, once so barren, is rapidly developing into a jungle; so this seems a good time to hack a way

through the undergrowth before it rises even higher.

Of course, not all works of the genre under consideration here are aimed at the same audience and not all cover precisely the same ground in precisely the same way. The massive *Glossary of Geology* (American Geological Institute: Washington DC, 1972) covers the whole range of the Earth sciences with some 33,000 entries which are generally short and highly technical. This is the working Earth scientist's bible and is likely to remain so, at least until the Institute summons up the courage to produce a new (third) edition. Not to be dismissed lightly, however, is the more humble *Penguin Dictionary of Geology* (D. G. A. Whitten and J. R. V. Brooks; Penguin: Harmondsworth, UK, 1972) which contains far fewer terms than the *Glossary* but devotes rather more words to each. Its language is perhaps best described as semi-technical, which makes the book useful to specialists

and comprehensible to students but hardly the sort of work the general reader would care to browse through.

The most serious objection to the Penguin dictionary is its weakness on geophysics, a gap that is filled to some extent by *A Dictionary of Earth Sciences* (S. E. Stiegeler; Macmillan: London and Basingstoke, 1976). I say "to some extent" because although the gap is undoubtedly filled, the relative shortness of the entries in the Stiegeler volume is not very helpful to those with no previous knowledge of the subject. This is a trap generally avoided by *Earth Resources: A Dictionary of Terms and Concepts* (D. Dinley et al.; Arrow: London, 1976) with its longer, more informative entries, although, as its title suggests, this book is narrower in scope.

If a distinction is to be made between dictionaries and encyclopaedias, all the books mentioned above, whatever their intended audience, would best be described as dictionaries; their entries, though generally not single-sentence definitions, are short and arranged alphabetically; and illustration, if any, is limited to a few line diagrams. By contrast, *The Planet We Live On: Illustrated Encyclopedia of the Earth Sciences* (C. S. Hurlbut; Abrams: New York; New English Library: London, 1976) comes closer to the popular idea of an encyclopaedia in that it contains hundreds of diagrams and photographs as well as numerous colour plates. Unfortunately, as I have had cause to remark before, this book contains so many inaccuracies that the whole stock is best left to rot in the publisher's warehouse. The point of mentioning it again here, however, is, first, to reiterate that warning and, second, to report that it is now possible to recommend to the general reader a book which, though similar in aim, is far superior in execution.

At the risk of being accused of using unscientific terminology, I can only describe *Planet Earth: An Encyclopedia of Geology*, edited by A. Hallam, as beautiful. It is nice to look at; colour photographs and/or diagrams adorn almost every page without making for simply a picture book with incidental text. It is accurate and authoritative; almost all the writers have been chosen from the ranks of those still active in the subject "because an understanding of the Earth depends on an appreciation of the fruits of recent research". It is for the most part well written—no mean achievement given the literary standards of a great many research workers. And it has a remarkably wide coverage, including some topics, such as engineering geology and the history of geology, which are all too often ignored.

It is also unconventional, in that it is arranged not alphabetically but in nine major sections each of which is subdivided into a series of closely related subjects. Thus, to take but one example, the section, "Processes That Shape the Earth", includes discussion of continental drift, weathering, earthquakes, diagenesis, and so on. The effect of this arrangement is to give the book a remarkable coherence. The pleasure of reading a text broken up into a series of 'terms' related only by the chance occurrence of their initial letters is strictly limited in my view, however useful such a book may be for reference. The delight of Hallam's approach is that it has produced not only a good work of reference (for the book has an excellent index) but also a more or less continuous text which cleverly directs the eye towards horizons beyond that originally sought. In short, it encourages the sort of pleasurable self-education that the disjointedness of an alphabetical arrangement can hardly begin to provide.

## Provocative but not profound

David Davies

*Ten Faces of the Universe*. By Fred Hoyle. Pp. 224. (Heinemann Educational: London, 1977.) £4.80.

THIS is the fourth book, to my knowledge, out of Cockley Moor this year and its publishers call it "perhaps Fred Hoyle's most provocative and profound book yet". Provocative it certainly is in parts—small parts—but profound it is not, and the lack of profundity only serves to devalue the provocation to something rather trivial.

The ten views of the universe are respectively God's, the physicist's, the mathematician's, the astrophysicist's, the expanding, the origin of the, nobody's (quantum mechanics), the geophysicist's, the biologist's and Everyman's. Hoyle has devoted an average of twelve pages of text to each; the rest of the book is made up of ten pages of chapter headings, ten more of pictures of people central to each chapter (with the relevance frequently left to the reader to work out); forty-seven pages of illustrations, many of them barely relevant; eight pages of star maps and coordinates; three pages listing details of all the elements; and four pages completely empty. The intended audience is American, to judge from the spelling and the baseball analogues,

So Hallam has nothing to fear from Hurlbut. He does, however, have more serious competition in the form of *The Physical Earth* (Mitchell Beazley: London, £12.50), one of the volumes in the much publicised Mitchell Beazley *Joy of Knowledge Library*. Despite the title, about one-half of this book is devoted to plants, animals and agriculture, although to the general reader the Earth science section will appear as attractive as *Planet Earth*, and for similar reasons. If forced to choose between the two books, I would go for Hallam, partly because the space devoted to the Earth sciences proper is greater and partly because the rather higher proportion of text to illustration somehow imparts to Hallam's work less of a coffee-table feeling. Besides, there is a considerable price differential. In comparison with books of similar format and physical quality the cost of *Planet Earth* is remarkably low.

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and presumably at the intelligent layman level.

The book starts on a poor note. Hoyle gives the impression he will discuss the place of a God in or outside the Universe. But he soon gets distracted into rambling anecdotes about his solution for Northern Ireland, how children learn mathematics, what his schooldays were like, and how Galois met his death. Thereafter the book is not free of its irritating diversions, but does follow a more orderly path. Hoyle certainly would not claim to be presenting other than thumbnail sketches of physics, mathematics and cosmology but there are some nice things therein, and insights that even a hardened scientist might find useful. The two chapters on geophysics and biology, not so close to Hoyle's lifelong interests, are accordingly more pedestrian.

Then we come to Everyman's Universe which is about energy, population and the collapse of society. Hoyle suggests that he could have devoted a whole book to these subjects. It is certain that a chapter tacked on the end is totally inadequate and fails to give much impression of deep and sustained thinking. Hoyle, like many others, is worried about population growth, but unlike most others he tries to discuss it in terms of simple-minded graphs and immensely accurate prediction. The world will collapse into a Dark Age in 2025, give or take a decade or two. How is this figure reached? We are not told. Why should productivity (whatever that means) and population collapse so dramatically? Because the Roman Empire did. Why should the

phenomenon "perhaps have a cycle time of about 500 years"? We are not told.

Hoyle the scientist has become Hoyle the crystal-ball gazer but retaining a pseudo-scientific veneer of graphs, cycles and so on. And his draconian proposal that we should stop all this growth by heavily taxing three-children

families just comes as a flashy bit of provocativeness which really advances serious thinking on problems in the real world very little.

In all, a strangely mixed book, at times charming, at times half-baked. □

David Davies is Editor of Nature.

## Cosmic gushers

Roman Znajek

*White Holes: The Beginning and End of Space.* By John Gribbin. Pp. 200. (Paladin: London, 1977.) £1.50; \$4.50.

A WHITE HOLE is like a black hole, but with time running backwards. Nothing can get out of a black hole, and nothing can get into a white hole. Whenever a black hole is formed, matter that is unable to withstand its own gravitational field collapses to form a point called a singularity. A white hole is *caused* by its singularity. The latter is best thought of as a remnant of the initial singularity from which the Universe began in the Big Bang. According to John Gribbin, if we are prepared to accept the existence of black holes then "the rest of the new astronomy inexorably follows", and that includes white holes. His book is intended to be a popular account of some of this new astronomy.

Now nobody knows for certain what a white hole singularity is going to do, and indeed nobody knows why it should be there at all. It is, however, not unreasonable to suppose that at some stage in its career it will start spewing things out and turn into a cosmic gusher, as Gribbins like to put it. Because the amount and nature of the gush is completely unknown, Gribbin has no difficulty in 'explaining' various highly energetic phenomena of extragalactic astronomy in terms of these cosmic gushers.

The conventional view is that quasars, radio-galaxies, and so on, derive their energy from matter in strong gravitational fields. For example, when gas spirals in towards a black hole very large quantities of heat are released through friction. There are reasons for expecting that kind of process to occur, even though there is much debate over the details. There are no reasons for expecting white holes to occur. Gribbin disagrees. According to him, the Big Bang is a white hole, and so there is every likelihood of there being others. But the universal Big Bang is a very different

phenomenon from the local white holes he thinks are responsible for quasars. What Gribbin cannot explain is why the Universe's initial singularity should have been deformed in such a way as to give rise to white holes.

Even if white holes did exist in the early Universe, they would have been very rapidly destroyed. In 1974, Douglas Eardley showed that white holes are unstable. A white hole attracts radiation and matter. But this material cannot get into it. It piles up on the surface of the hole, getting continuously accelerated. An exponentially strengthening 'blue sheet' is formed, which twists round the gravitational field and turns the white hole into a black hole. The black hole remains, because black holes are stable. The process is very rapid, and only ridiculously large white holes would survive into the present epoch.

It is interesting to see how Gribbin tackles this problem. First of all, he refers to Eardley's work as a "suggestion". He then says that "better physics is needed to deal with the situation". This is correct in so far as we don't know why the white hole is there, but given its existence Eardley's argument is immediately applicable. Gribbin finishes by claiming that this difficulty "is more than compensated for by the amazing discovery in the mid-1970s that black holes can explode". This is the cue for five pages on the Hawking process, which is interesting in itself but completely irrelevant to Gribbin's cosmic gushers.

The possession of unconventional ideas does not disqualify a person from writing a popular book. But he should at least give a good account of the orthodox view. Gribbin doesn't. The saddest thing about his book is that his astronomy is old. It is five years or so out of date. In the sixties astronomers were often baffled by the objects they saw through their telescopes, and were prepared to believe in all kinds of curious explanations. It now seems that there is no need to invoke unlikely objects such as white holes in order to understand quasars. White holes are old-fashioned. □

Roman Znajek is an SRC Postdoctoral Fellow at the Institute of Astronomy, Cambridge, UK.



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# Amplifying by stimulated emission

Harold Weaver

*Celestial Masers.* By A. H. Cook. Pp. 135 (Cambridge University: New York, London and Cambridge, 1977.) £7.50.

ANY new technological development that makes possible astronomical observations in a previously unexplored spectral range never fails to have a strong impact on the science. Radio astronomy (which covers the spectral range from about one millimetre to longer wavelengths of many meters) provides many examples. The science itself was started accidentally by Karl Jansky in 1931 when he discovered radio radiation from the Galaxy. It has developed rapidly since the late 1940s, providing an extraordinarily rich harvest of new information and broadening enormously our view of the nature of the Universe. It first demonstrated the existence of synchrotron radiation, generated by relativistic electrons spiraling in magnetic fields in our own Galaxy and others.

Quasars, pulsars, and the all-pervading 3 K black-body radiation that is the relic of the original big bang that marked the start of the expanding Universe, are all discoveries of radio astronomy. The first spectral line in the radio range (the radiation arising from neutral hydrogen at a wavelength of 21 cm) was discovered in 1951, and the first molecular line in the radio range (originating in the hydroxyl radical OH) was observed in 1963.

Since then, radio astronomy has greatly changed astronomers' views of the chemistry of the interstellar medium. Radio astronomy has shown that polyatomic molecules, earlier thought to be highly improbable if not impossible in the interstellar medium, do, in fact, abound in the dark cool clouds of interstellar space. Radio spectral lines from several dozen different polyatomic molecules, some containing up to nine atoms, have been observed.

An extraordinary feature of the radio lines of the OH molecule was discovered in the course of a series of observations made at the Hat Creek Observatory of the University of California at Berkeley during 1964–65. The Hat Creek programme called for observations of all likely radio sources in which the OH molecule might appear. Many clouds containing OH were found, but in a few cases in which

dark clouds were associated with regions of ionised hydrogen excited by hot, ultraviolet emitting stars, the behaviour of the four spectral lines in the multiplet observed was totally anomalous.

The shapes and strengths of the four lines of the multiplet seemed to have little or no relationship to one another, a situation completely out of keeping with theoretical predictions. The lines were mixtures of broad absorption and numerous sharp emission components. Subsequently, it was found that in some instances the sharp emission features were polarised (some linearly, some circularly), and that some features varied in intensity on the timescale of a few weeks. The emission components originated in regions of the dark clouds having angular diameters far smaller than could be resolved by any single radio telescope. Later observations showed that the OH emission came from areas less than  $10^{-2}$  arcs in size.

It is an interesting historical sidelight that, while these observations were in progress at Hat Creek, similar observations were being made at the Harvard Radio Astronomy Observatory, where the same peculiarities were independently discovered. Neither group knew of the work of the other. By chance, the discovery was published first by the Berkeley investigators.

The peculiarities of the OH emission combined with the intensity of the radiation and the small signs of the sources from which it originated, were clearly inconsistent with spontaneous emission from a gas in thermal equilibrium. Amplification by stimulated emission must be taking place. Natural masers (microwave amplification by stimulated emission of radiation) in astronomical objects were thus accidentally discovered a few years after masers were invented on Earth. An interesting speculation will always remain as to how the theory of masers might have developed if the astronomical observations had come first.

In his brief 135-page monograph Professor A. H. Cook deals with the observation and interpretations of these intriguing astronomical objects. The topic has, of course, grown greatly in the 12 years since the first discovery. It is now known that not only does the OH group but also the H<sub>2</sub>O and other molecules, show maser action. Theories of "how it works" are numerous.

In the preface to his book Professor Cook states: "I have not presumed to write a book that will sum up the subject and give an explanation. Rather I have tried to bring out what is not known; no doubt I shall provoke disagreement, and if that disagreement leads to new knowledge and insight,

much of my purpose will have been achieved. This is meant to be an interim report and a stimulus for further study." Professor Cook has very successfully achieved his goal. His summaries and concluding remarks should prove stimulating to both seasoned workers in the field and to those who may wish to enter the field.

Of the six chapters in the book, Professor Cook devotes the first two to a short account of selected spectral lines in radio astronomy and a very brief review of those principles of molecular structure and spectroscopy required for discussion of celestial masers. He illustrates the topics covered with examples from OH and H<sub>2</sub>O sources.

The third and longest chapter provides a very adequate summary of observations of maser action for OH and H<sub>2</sub>O sources. Here, Professor Cook has steered a straight and true course, emphasising and summarising those features that are important for a basic understanding of the topic and foregoing the very large amount of detail found in extensive research papers.

In explaining the amplification of stimulated emission (chapter 4), the author adopts a phenomenological point of view and avoids a general quantum mechanical treatment. He provides clear brief derivations or explanations of the principal aspects of astrophysical masers, such as the equations of transfer and rate, the meaning of saturated and unsaturated masers, gain, linewidth, and the like. But the author's major thrust in this chapter is to point out the gaps in current knowledge. "We do not assuredly know", he writes, "how to account for the small angular sizes of sources, the isolated distribution of individual sources, the single sense of polarisation in hydroxyl, and, again in hydroxyl, radiation in only one transition. Possibly the greatest lack is the absence, so far, of any but the sketchiest account of lasers varying with time".

After reviewing quite thoroughly in chapter 5 the many schemes that have been suggested for pumping, that is, for inverting the populations in OH and H<sub>2</sub>O sources to produce a maser, Professor Cook goes on in chapter 6 to provide an analysis and interpretation of maser radiation. Here he attempts "to see how far the sources can be accounted for by amplification of stimulated emission and to use the analysis to set conditions that must be fulfilled by any pumping mechanism. Other clues to pumping mechanisms may be provided by the statistical properties and associations of maser sources". The chapter is a very interesting one in which the author enumerates various classes of problems

remaining to be solved; and, in some instances, he suggests bases for their solution. Observational classification schemes of OH sources are paired with theoretical pumping schemes in a most interesting way. Finally, Professor Cook tries briefly to fit celestial masers into a wider picture of the star formation process.

*Celestial Masers* is a book that many will find interesting and stimulating. I

highly recommend it. Unfortunately, it does contain a few typographical errors in both references and equations, but the reader will readily recognise and easily correct these very minor blemishes. □

*Harold Weaver is Professor of Astronomy at the University of California at Berkeley, and was the Founder-Director of the Radio Astronomy Laboratory at Hat Creek.*

## Nuclear shapes and sizes

C. J. Batty

*Nuclear Sizes and Structure.* By R. C. Barrett and Daphne F. Jackson. (Clarendon: Oxford, 1977.) Pp. 566. £17.50.

In his classic paper on the scattering of alpha particles at large angles, Rutherford wrote: "Considering the evidence as a whole, it seems simplest to suppose that the atom contains a central charge distributed through a very small volume, and . . .". And so we had the first insight into the structure of the atom with its central charged nucleus, later shown to consist of (uncharged) neutrons and (charged) protons, surrounded by a cloud of electrons. Since then, our knowledge of the constituents and the structure of the nucleus has advanced rapidly, but this very sentence of Rutherford's also poses more detailed questions. How big is the nucleus? What shape does it have? How are the constituents and the electric charge distributed? And so on.

The study of nuclear sizes, in its widest sense, has remained since those early days at the core of nuclear physics and of studies on the structure of the nucleus. The success, or otherwise, with which nuclear models can make meaningful predictions as to the distribution of charge and nuclear matter in the nucleus has always been a sensitive test of their validity. The study of nuclear shapes and sizes too has always encompassed a very wide range of topics. The charge distribution, and hence the proton distribution, can be sensed with the electron or the muon through the electromagnetic interaction, and we now have quite detailed information available from measurements of electron scattering and with muonic atoms. Study of the matter distribution which is necessary to provide the neutron distribution, requires the use of strongly interacting probes such

as the proton, alpha particle, pion, kaon, and so on; and a much wider range of nuclear scattering and interaction processes are involved. As a result, our knowledge is much less certain and in certain cases ambiguous or downright conflicting.

It is now 16 years since the monograph on *Nuclear Sizes* by L. R. B. Elton appeared, and the intervening years have seen dramatic advances in our knowledge of nuclear shapes and sizes and in the techniques used for their study. This book by Roger Barrett and Daphne Jackson of the University of Surrey is therefore particularly welcome and provides a long needed successor to the earlier monograph.

Over the past few years, it has become clear that the different types of experiments used to look at nuclear sizes probe different regions of the nuclear matter or charge density and hence are sensitive to different parameters or combinations of parameters of the form chosen for the distribution. It has also led to the so-called 'model-independent' analyses in which there is no rigid *a priori* choice of the form for the nuclear distribution and in which the uncertainties in the derived distribution are more clearly seen.

The first chapter of the book, then, deals with these introductory ideas. Various distribution functions and form factors are defined and the ideas of radial parameters and moments introduced. Some useful comparisons are also made between the numerical parameters calculated from a range of neutron and proton distributions.

It might be concluded, so far, that all the advances of recent years have been on the experimental front. This is by no means so, and the second chapter deals with the various current theories of the nuclear matter distribution. After some introductory ideas, the single-particle model is discussed; in this model individual particles move in an averaged phenomenological nuclear potential. As this potential is, in fact, derived from the interactions of all the nucleons in the nucleus, the model lacks self-consistency. This problem is avoided by using the Hartree-Fock method; and an extensive review

of recent calculations is given. Macroscopic models in which the emphasis is on bulk nuclear properties are next discussed, particularly the Thomas-Fermi approximation and the hydrodynamical model; and the chapter then concludes by considering the especial features of calculations for deformed nuclei. Results from the various types of calculations are compared in a number of useful tables and graphs throughout the chapter.

As already mentioned, measurements of electron scattering and with muonic atoms are used to obtain information about the charge distribution in nuclei; and these topics are discussed in the next two chapters. After considering the motion of electrons and muons in an electromagnetic field, the scattering of electrons by the nuclear charge is discussed. Analyses to obtain the charge distribution using either a simple parameterised model or with a 'model-independent' method are next described, together with a brief discussion of the problems raised by analyses for deformed nuclei. Large angle magnetic scattering is also considered, followed by a discussion of dispersion, radiative and straggling corrections.

For muonic atoms, the chapter moves quickly into a discussion of 'model-independent' analyses of the X-ray spectra, the energy levels for deformed nuclei, magnetic hyperfine structure, and isotope, isomer and isotope shifts. Radiative corrections are particularly important in muonic atoms and until recently were the subject of considerable controversy when measurements for high transitions seemed to indicate a disagreement between experimental results and calculations using quantum electrodynamics. Happily, this problem now seems to be resolved and a review of these topics completes the chapter.

The use of optical and X-ray spectral methods to measure isotope and isomer shifts is only very briefly touched on in the next chapter (just ten pages long). This is unfortunate as the topic is of considerable current interest, particularly in view of the increasing use of laser techniques. Also, the following chapter on experimentally derived charge distribution shows that quite a large body of information has been obtained in this way. This latter chapter, as well as including tables on isotope and isomer shifts, also includes tables on charge distributions for a very wide range of nuclei, including deformed nuclei. Inevitably, such information rapidly becomes out of date and the serious researcher will need to go back to the original literature or to other, more extensive, data compilations. Nevertheless, the tables present a great deal of

useful information in a very compact form and include an extensive set of references to enable the reader to dig deeper.

As indicated, the study of the nuclear matter distribution involves the use of strongly interacting projectiles and the derivation of the required information from nuclear scattering or reaction experiments. These topics, which are dealt with in the next two chapters, are inevitably complicated and it is not too surprising that together these two chapters account for almost half the book.

Nuclear scattering is dealt with first. After an introduction to partial wave methods of analysing scattering from a potential and to the various approximations which are used, the formal theory and general properties of the optical model are dealt with. This is followed by a review of the information gained from analyses of nucleon-nucleus scattering. This review is split into three subsections, covering different energy regions, and includes many tables of optical model potentials and parameters. An equally complete review of the scattering of composite projectiles and of the nuclear size information which can be obtained from experiments then follows.

The chapter on nuclear reactions starts with an extensive discussion of inelastic scattering both to resolved final states and then to summed final states; the former gives information principally about particular spectroscopic states and the latter about average nuclear properties. There follows descriptions of the theory and analysis of transfer, knock-out and photonuclear reactions, and finally of alpha decay. Inevitably, any discussion of nuclear reactions also involves problems related to nuclear reaction mechanisms and to the structure of nuclei. The chapter does, however, pay particular attention to the information about nuclear sizes and other closely related nuclear properties which can be obtained in this way.

It was originally thought that measurements on hadronic atoms would be capable of giving clear information on nuclear matter distributions. It turns out, however, that here nature has not been particularly kind. In the case of pionic atoms, the interaction of the pion with the nucleus is complicated by the presence of dominant  $p$ -wave processes, whereas for kaonic atoms the resonance in the kaon-proton system again complicates the analysis of experimental data. It is still hoped, however, that, with our growing understanding of these interactions, it may be possible to obtain nuclear size information; these topics therefore are discussed in chapter 9 of the book.

The final chapter concentrates on processes capable of giving information about the neutron distribution directly rather than through separate measurements of the proton and matter distributions. Available methods for looking at the excess neutrons include studies of Coulomb energy differences or of charge-exchange reactions. Alternatively, the scattering of pions can be measured at energies at which they interact preferentially with neutrons. Pion production, or the interactions of stopping kaons, can also be used, since in both these cases certain processes can only occur through interactions with neutrons.

This chapter-by-chapter review will have indicated the very wide range of topics covered by the book, all of which are related in some way to the question of nuclear sizes. In view of this broad coverage there is inevitably some unevenness in the treatment of the various topics. How much this matters is a matter for individual judgment, which each reader will have to decide for himself in the light of his own special interests.

The book contains a large number of tables and figures of useful data. As already mentioned, a few of the tables will rapidly become out of date. Nevertheless, they comprise a useful collection of information related to nuclear sizes. The book also contains a list of approximately 1,500 references which fill the last 50 pages of the book and which are frequently referred to throughout its pages. This makes the volume particularly useful to the specialist, who will wish to refer to the original literature. It does, however, create the air of an extended review article rather than that of a definitive text which might appeal more to the student approaching the subject for the first time. To some extent, this is due to the authors' determination to include the most recent developments.

Finally, as the authors themselves point out, the past few years have seen an enormous growth in our knowledge of nuclear sizes and in the range of experiments used for their study. The book provides an excellent review of the situation as it was at the time of writing in 1975. The next few years are likely to see an equally rapid progress in both experimental work and in theoretical calculations. It is also likely to see an ever-increasing range of methods in atomic, nuclear and particle physics being used to study the sizes and shapes of nuclei. □

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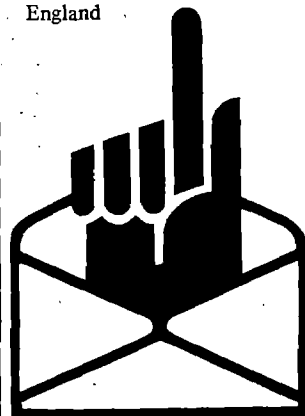
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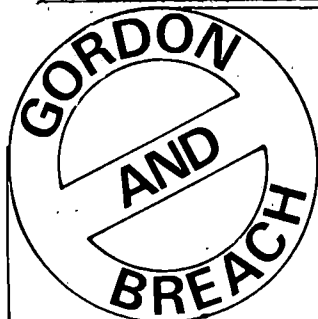
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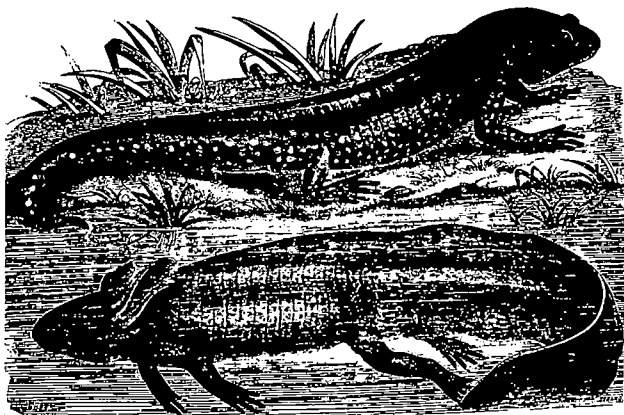
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# review article

## Nature of lethality of *t* mutations in embryos

Linda R. Wudl\* Michael I. Sherman\* & Nina Hillman†

*Mutations in the genes of the T complex in mice can lead to death at various stages of embryogenesis. It has been suggested that the T genes specify cell surface antigens which are essential for normal organisation of certain cells within the embryo. But, there is now evidence that at least some of these T complex mutations are simply generalised cell lethals.*

THE set of genes in the mouse known as the 'T complex' has been studied extensively for many years. However, there is still disagreement on both the genetics and the biochemistry of this system. The confusion arises in attempts to explain how apparently single genetic alterations can result in multiple phenotypic effects. Such alterations in the T region, conventionally referred to as recessive (*t*) mutations, may cause (1) death of homozygous embryos at characteristic stages, (2) taillessness when combined with the Brachyury (*T*) mutation in a compound heterozygote (*T/t*), (3) non-Mendelian inheritance from the male parent, (4) suppression of recombination over a rather large region of chromosome 17 (extending, in the case of *t<sup>w5</sup>*, from the centromere to well beyond the H-2 region) and, (5) male sterility when combined with other, complementary, *t* mutations (*t<sup>x</sup>/t<sup>y</sup>*)<sup>1,2</sup>. Present knowledge of the genetics of the T region indicates that although a single mutagenic event may have resulted in what is called the '*t* mutation', a large portion of the genome and multiple loci are affected<sup>1,2</sup>. We shall discuss only the lethal effects of mutation in the T region and the relationship between the *t* genes (or gene) and the programme for early embryonic development.

Two theories have been put forward to explain the lethality of *t* mutations. The first, and most widely quoted, of these proposes that the primary lesion is an alteration at the surface of certain embryonic cells which prevents the cell-cell recognition and interaction required for tissue formation ('organisational failure' hypothesis)<sup>1-6</sup>. The affected embryo becomes disorganised and is resorbed in the uterus. The second theory is simply that, at least for some *t* mutations, all cells of the embryo are adversely affected and that death of the embryo is due to generalised cell lethality<sup>7-11</sup>.

In earlier studies<sup>11</sup>, we tested the organisational failure hypothesis by monitoring the behaviour of mutant embryos *in vitro*. In our culture conditions<sup>12,13</sup>, the organisation and interactions of different cell types are less important for cell survival than they are *in utero*. This is documented by our success in establishing a number of cell lines from cultured blastocysts even though several cell types (for example, embryonic ectodermal derivatives) either degenerate soon after formation or are never produced. In fact, the cell types that develop most reliably in our cultures are extraembryonic endoderm and trophoblast<sup>13</sup>; these are the very cell types that seem to survive longest *in utero* in conceptuses homozygous for every lethal *t* allele<sup>2</sup> with the possible exception of *t<sup>w73</sup>* (ref. 14). Consequently, our *in vitro* techniques should have enabled us to recover those cells from homozygous *t*-mutant embryos that are presumably not directly affected by the mutation. However, when we tested homozygous *t<sup>w5</sup>* mutant embryos<sup>11</sup>, we found that none of the cells was capable of surviving *in vitro*, and indeed, most of the

cells had died at the time of the normal lethal period for *t<sup>w5</sup>/t<sup>w5</sup>* embryos *in utero* (7-10th day of gestation<sup>15</sup>). Furthermore, when pairs of embryos are combined to form chimaeras, homozygous mutant cells in the mosaic embryos do not seem to be rescued by contact with their normal neighbouring cells; in other words, the *t<sup>w5</sup>* mutation behaves as a generalised cell lethal and not as an organisational lethal<sup>11</sup>.

Investigators studying the extrauterine development of embryos bearing two other recessive *t* mutations, *t<sup>6</sup>* (ref. 16), which causes death earlier than *t<sup>w5</sup>*, and *t<sup>w18</sup>* (ref. 17), which leads to embryonic failure at later stages, have come to conclusions different from those we have drawn on the basis of experiments with *t<sup>w5</sup>*. Erickson and Pedersen<sup>18</sup> reported that the inner cell mass (ICM) of presumptive *t<sup>6</sup>/t<sup>6</sup>* blastocysts showed a rapid degeneration *in vitro* while the trophoblast remained viable. They concluded, therefore, that the adverse effects of the *t<sup>6</sup>* mutation were selective for the ICM moiety of the embryo. Artzt and Bennett<sup>19</sup> transplanted 9th d presumptive *t<sup>w18</sup>/t<sup>w18</sup>* embryos under the testis capsule of adult mice and proposed, from analyses of the resultant growths, that ectodermal derivatives of these embryos were capable of surviving whereas mesodermal elements failed to develop. We report here the results of our studies on the development of *t<sup>6</sup>/t<sup>6</sup>* blastocysts both in culture and in an ectopic site. These studies were undertaken to determine whether any cells from the mutant embryos stand a better chance of survival in these experimental conditions than they do *in utero*. We have also assessed the behaviour of *t<sup>w18</sup>/t<sup>w18</sup>* embryos *in vitro*.

### Behaviour of *t<sup>6</sup>/t<sup>6</sup>* embryos *in vitro*

An analysis of the male transmission frequency of the *t<sup>6</sup>* mutation is shown in Table 1a. By the use of these data, we can deduce the percentage of blastocysts with the *t<sup>6</sup>/t<sup>6</sup>* genotype in our cultures (Table 1b). The ICM cells in *t<sup>6</sup>/t<sup>6</sup>* embryos behave under our culture conditions as observed by Erickson and Pedersen<sup>18</sup> in their experiments: by the 7th EGD (equivalent gestation day, that is the age of the embryos had they been left *in utero*), all ICM cells in 35% of the embryos had disappeared, suggesting that more than 80% of the *t<sup>6</sup>/t<sup>6</sup>* embryos contained only trophoblast. By the 8th EGD, all homozygous mutant embryos seem to have lost their ICM (Table 1c).

When we examined the trophoblast moiety of presumptive *t<sup>6</sup>/t<sup>6</sup>* embryos, however, we could see that they too had been affected by the mutation. In all control blastocyst outgrowths containing an ICM, and in the occasional blastocyst outgrowth which had lost its ICM by the 8th EGD, the trophoblast cells had proceeded to polyploidise and become giant. By contrast, the trophoblast cells of presumptive *t<sup>6</sup>/t<sup>6</sup>* blastocyst outgrowths did not become giant<sup>2</sup>.

In a subsequent series of experiments, we generated trophectodermal vesicles from disaggregated blastomeres of normal and *t<sup>6</sup>/t<sup>6</sup>*

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**Table 1** Transmission frequency distortion of the  $t^6$  allele and behaviour of blastocysts bearing the  $t^6$  mutation in culture

a Transmission frequency of the $t^6$ mutation							
Genotype at T region of animals mated female male (+/+) × ( $T/t^6$ )		Number of offspring  1,656	Number of offspring with normal tails  1,318		Transmission frequency of $t^6$  79.6%		
b Expected proportions of genotypes in mixed embryo populations							
Cross	Genotype at T region of animals mated female male	% of embryos with genotype					
Control	(+/+) × (+/ $t^6$ )	+/+	+/t <sup>6</sup>	$t^6/t^6$			
Experimental	(+/ $t^6$ ) × (+/ $t^6$ )	20.4	79.6	—			
		10.2	50.0	39.8			
c Survival <i>in vitro</i> of ICM cells in embryos carrying the $t^6$ mutation							
Genotype at T region of animals mated	Number of blastocysts cultured	7th EGD*		% of ICMs surviving 8th EGD		11th EGD	
(+/+) × (+/ $t^6$ )	432	observed	expected†	observed	expected†	observed	expected†
(+/ $t^6$ ) × (+/ $t^6$ )	503	96.3	—	95.4	—	91.2	—
		65.2	58.0	54.5	57.4	47.4	54.9

In *a*, to determine transmission frequency, wild-type (SWR/J) females were mated with tailless males. Offspring of genotype +/ $t^6$  were normal-tailed (and were used in the experiments in *c*), while T/+ progeny were short-tailed. Part *b* of the table, based on the data in part *a*, gives the expected proportions of genotypes of the embryos used in *c*. In *c*, embryos were removed at the blastocyst stage, on the fourth day of pregnancy, washed, and cultured individually in NCTC-109 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics as described by Wudl and Sherman<sup>11</sup>. They were scored for the presence or absence of inner cell mass derivatives with phase contrast optics.

\*EGD, equivalent gestation day, that is, the age of the embryos had they been left *in utero*.

†Expected values if ICM cells are absent in all  $t^6/t^6$  embryos and if ICMs of heterozygous and wild-type embryos in the control and experimental populations survive with the same frequency.

mutant embryos<sup>20,21</sup>. Generally, at a time when control embryos have formed mature blastocysts, trophoblastic vesicles contain only a single layer of trophoblastic cells, that is they are devoid of ICM cells. When trophoblastic vesicles are placed in the appropriate culture medium, they become attached to the substratum and give rise to outgrowths of trophoblastic cells which are capable of differentiation as determined by morphological and biochemical criteria<sup>21,22</sup>. We studied one of these criteria, polyploidisation, in outgrowths from normal and presumptive  $t^6/t^6$  trophoblastic vesicles by monitoring the nuclear diameters of trophoblastic cells. (We have shown elsewhere<sup>23,24</sup> that DNA content in polyploid trophoblastic cells is proportional to nuclear size.) Approximately 40% of trophoblastic vesicles from the experimental cross (see Table 1) gave rise to trophoblastic cells which possessed few or no giant nuclei (for example, Fig. 1, *a*, *c*, *e*, *g*) before death on or about the 12th EGD. On the other hand, in the control cross, which yields only heterozygous and wild-type embryos, all trophoblastic vesicles gave rise to polyploid trophoblastic cells by the 8th EGD (for example, Fig. 1, bottom photos). Non-mutant trophoblastic cells survived longer than the arrested cells in the  $t^6/t^6$  trophoblastic outgrowths (Fig. 1, *g*, *h*). A detailed quantitative analysis of these experiments, to be presented elsewhere (L.W. and M.I.S., in preparation), indicates that trophoblastic vesicles from normal (+/ $t^6$  and +/+) embryos in the experimental cross polyploidise to the same degree as their counterparts in the control cross.

**Table 2** Incidence of haemorrhagic nodule formation in kidneys bearing implants of blastocysts from control and experimental crosses

Cross	No. of blastocysts implanted	No. of kidneys containing nodules	% observed	% expected
Control (+/+) × (+/ $t^6$ )	68	43	63	—
Experimental (+/T $t^6$ ) × (+/ $t^6$ )	63	28	44	38

Fourth day blastocysts were removed from uteri and placed in culture. Within six hours, blastocysts were transplanted individually under the capsule of the left kidney of adult male siblings. Kidneys were scored macroscopically for the presence of haemorrhagic nodules. The percentage of growths observed in the control and experimental crosses was significantly different by  $\chi^2$  analysis ( $\chi^2 = 7.25$ ,  $P < 0.01$ ). The percentage of growths in the experimental cross observed and expected (assuming that all  $t^6/t^6$  embryos will fail to produce growths and that the transmission frequency for the  $t^6$  allele is 79.6%) was not significantly different ( $\chi^2 = 0.74$ ,  $P = 0.5$ ).

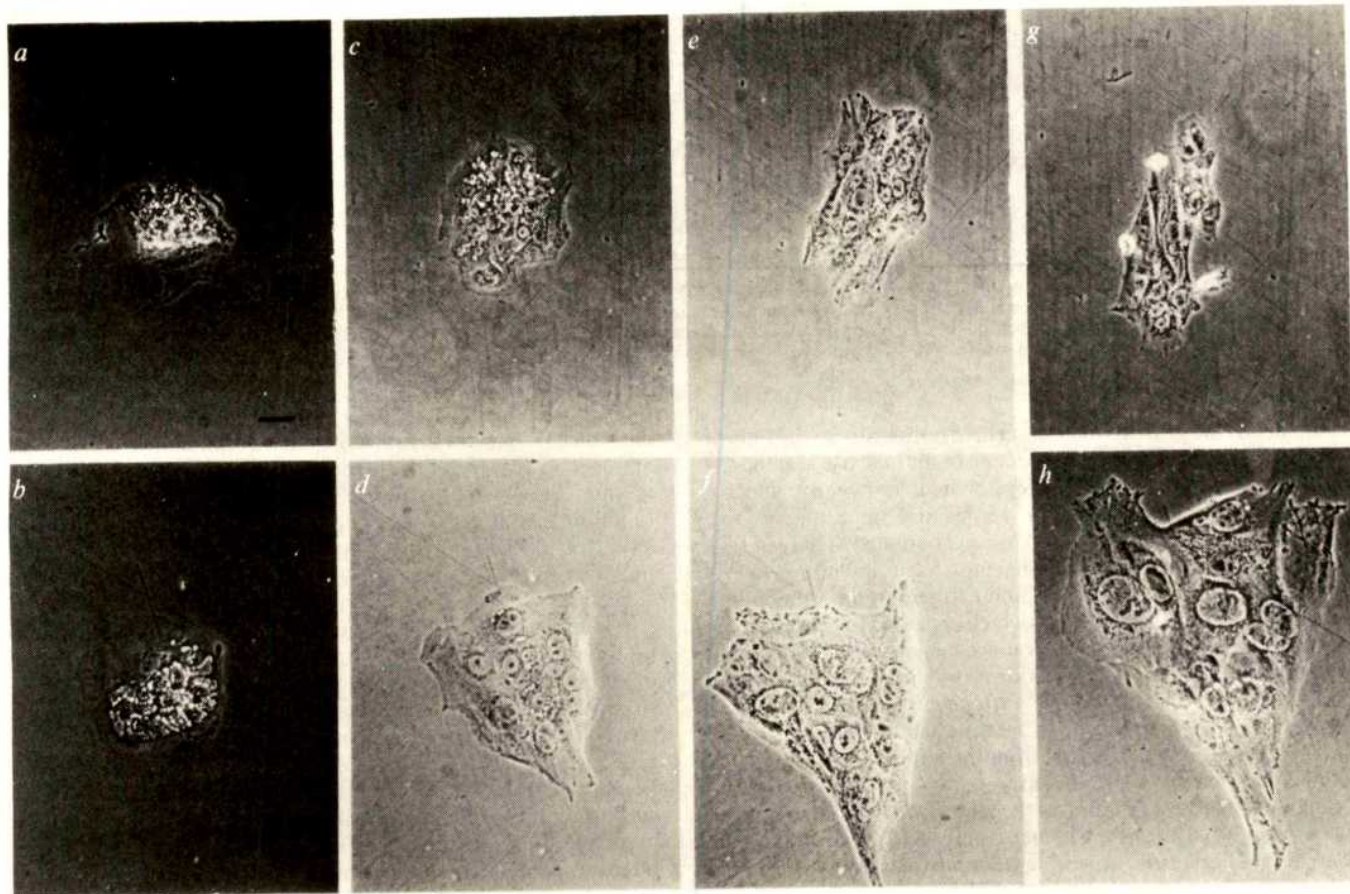
The obvious interpretation of these results is that trophoblastic cells are affected by the  $t^6$  mutation, contrary to the report of Erickson and Pedersen<sup>18</sup>; furthermore, the arrest of  $t^6/t^6$ , but not normal, cells in these experiments renders it unlikely that mutant trophoblastic cells fail through a lack of interaction with other cells, since there are no cells other than trophoblastic in trophoblastic vesicle outgrowths. Organisational failure is thus an inadequate explanation of the adverse effects of the  $t^6$ , as well as the  $t^{w5}$ , mutation.

We have now begun to monitor  $t^6$  mutant trophoblastic cells for production of other markers of differentiation. We found (L.W. and M.I.S., in preparation) that presumptive  $t^6/t^6$  trophoblastic cells possess plasminogen activator activity during the period in which normal trophoblastic cells produce and secrete this enzyme<sup>22,25</sup>. They also convert pregnenolone to progesterone, indicating that they possess the enzyme  $\Delta^5$ , 3 $\beta$ -hydroxysteroid dehydrogenase, as do normal trophoblastic cells<sup>24,26</sup>. These results suggest that the individual elements of the developmental programme of trophoblastic cells may be regulated separately, and that mutation in the T region may interfere selectively with some developmental processes, but not others.

### Behaviour of $t^6/t^6$ embryos in an ectopic site

Although all cells from  $t^6/t^6$  embryos are adversely affected by the mutation in culture, some cells from presumptive  $t^{w18}/t^{w18}$  embryos implanted under the testis capsule survive for times longer than the gestation period<sup>19</sup>. It is, therefore, possible that  $t^6$  and  $t^{w18}$  mutations differ, either in type or in severity. Alternatively, one might argue that ectopic sites provide a rich environment for growth of embryonic cells, allowing proliferation of genotypically mutant cells that do not require wild-type T-gene products for survival and growth, whereas our culture conditions might be detrimental to all homozygous  $t$ -mutant cells. To distinguish between these possibilities, we implanted blastocysts from  $t^6$  control and experimental crosses under kidney capsules of immunologically appropriate host mice, and inspected the kidneys of these animals for ectopic growths 14 d later (Table 2). We found that the blastocysts from the experimental cross gave rise to a significantly lower proportion of growths visible under the dissecting microscope as haemorrhagic nodules when compared with blastocysts from the control cross. In fact, the observed number of growths was not significantly different from that expected if all  $t^6/t^6$  embryos had failed to survive the 14-d period under the kidney capsule. The size of the nodule varied from one





**Fig. 1** Behaviour of mutant ( $t^6/t^6$ ) and normal ( $+/+$ ,  $+/t^6$ ) trophoblast cells developing from trophoblastic vesicles. Trophoblastic vesicles were generated by disaggregating 4- and 8-cell embryos<sup>21</sup> from control ( $+/+ \times +/t^6$ ) and experimental ( $+/t^6 \times +/t^6$ ) crosses and culturing individual blastomeres from 4-cell embryos and pairs of blastomeres from 8-cell embryos in Whitten's modified ovum culture medium<sup>33</sup>. After 48 h (5th EGD), structures scored as trophoblastic vesicles were placed individually in serum-supplemented NCTC-109 medium<sup>11</sup>. Trophoblast cells outgrowing from a presumptive mutant ( $a, c, e, g$ ) and a normal ( $b, d, f, h$ ) trophoblastic vesicle were photographed sequentially on the 7th ( $a$  and  $b$ ), 8th ( $c$  and  $d$ ), 9th ( $e$  and  $f$ ) and 11th ( $g$  and  $h$ ) EGD using phase contrast optics. Magnification is the same in all photographs. Scale bar (in  $a$ ), 50  $\mu$ m.

implant to the next, but the range of sizes was the same in the experimental and control crosses.

Approximately half of the kidneys receiving implants of blastocysts were processed histologically and analysed by light microscopy (Table 3) to determine whether we might have missed viable  $t^6/t^6$  ectopic growths either because they were much smaller than those caused by normal embryos or because they were devoid of functional trophoblast cells and, therefore, non-haemorrhagic. In either case, the growth might not be discernible when the kidneys were examined macroscopically. The histological studies also enabled us to compare growths from experimental and control blastocysts to determine whether the growths from one cross might contain cell types not present in growths from the other. We found, however, that virtually all kidneys judged to be positive by the presence of a macroscopic haemorrhage contained extravasated blood, large numbers of giant trophoblast cells, both mononucleated and multinucleated, and extraembryonic endodermal cells.

In several of the explants, the endodermal cells were associated with an amorphous eosinophilic material that has been described by other investigators<sup>27-29</sup> as Reichert's membrane.

No other recognisable embryonic tissues or organs were found in any of the haemorrhagic nodules at the time of autopsy. However, the extensive growth of trophoblast cells and identification of extraembryonic endoderm attest to the presence in the growths, at least initially, of ICM cells<sup>29,30</sup>. In all of the growths, some scattered necrotic cells were present, and these might have been of ICM origin. Fibroblastic cells were commonly observed around the periphery of the growths, and, less frequently, in the central blood cavity. It is assumed that these cells were of maternal origin. In about 40% of the cases in both experimental and control crosses, the trophoblast cells were actively invasive and phagocytosed kidney cells could be seen in the giant cell cytoplasm; the

**Table 3** Histologic analyses of kidneys receiving implants of normal and mutant ( $t^6/t^6$ ) blastocysts

Cross	No. analysed	Macroscopic positives		Macroscopic negatives	
		No. containing trophoblast and extra-embryonic endoderm cells	No. containing Reichert's membrane-like material	No. analysed*	No. containing growths
Control ( $+/+$ ) $\times$ ( $+/t^6$ )	16	15†	7	15	0
Experimental ( $+/t^6$ ) $\times$ ( $+/t^6$ )	17	17	6	15	1

After the kidneys referred to in Table 2 were inspected macroscopically for the presence of growths, they were placed in neutral buffered formalin, embedded in paraffin and serially sectioned (8  $\mu$ m). After dehydration in a graded series of alcohol, the sections were stained with Delafield's haematoxylin and eosin.

\*Entire kidneys were prepared as described above and every section was scanned with low power optics.

†One nodule, which was very large, was only partially sectioned. Those sections inspected contained only blood cells and extraembryonic endoderm cells.



**Table 4** Survival *in vitro* of embryos carrying the  $t^{w18}$  mutation

Cross	Expected % of homozygous mutant embryos	No. of blastocysts cultured	% of blastocysts surviving 22–24th EGD	
			observed	expected*
Control (+/+) × (+/ $t^{w18}$ )	0	404	55.0	—
Experimental (+/ $t^{w18}$ ) × (+/ $t^{w18}$ )	28.7†	402	51.5	39.2

The +/ $t^{w18}$  mice were generated by selecting normal-tailed offspring from the cross of SWR/J females (wild-type in the T region) with  $T/t^{w18}$  males (obtained from Dr Karen Artzt, Sloan Kettering Institute for Cancer Research).

\*Expected incidence if all  $t^{w18}/t^{w18}$  embryos fail to survive the gestation period *in vitro*.

†The proportion of homozygotes is low for  $t^{w18}/t^{w18}$  embryos because the  $t^{w18}$  allele does not show substantial transmission frequency distortion<sup>17</sup>.

remainder of the growths did not seem to be invasive at the time of autopsy, but rather caused a depression of the host tissue at the site of transplantation. Of 30 kidneys scored as negative macroscopically, all but one proved to be negative upon microscopic examination. The exceptional kidney contained a large, subcapsular growth, which was non-haemorrhagic and non-invasive. It lacked trophoblast giant cells and extraembryonic endoderm, containing instead epithelial cells which appeared to be embryonic in nature, some of which were arranged cylindrically, reminiscent of neural tubes. Although this kidney was in the 'experimental' group, we have no way of knowing whether it had received a  $t^6/t^6$ , a +/ $t^6$  or a +/+ blastocyst. In fact, we cannot say for certain whether this growth originated from the implanted blastocyst or whether it developed spontaneously.

The most likely explanation of these observations is that normal (+/+, +/ $t^6$ ) blastocysts, whether from the experimental or the control cross, give rise to the same kinds of growth under the kidney capsule, while all cells from  $t^6/t^6$  blastocysts die within the two week incubation period.

### Behaviour of $t^{w18}/t^{w18}$ embryos *in vitro*

As the results in Table 4 illustrate, over 50% of individually cultured control blastocysts from a population consisting of approximately equal numbers of +/+ and +/ $t^{w18}$  embryos give rise to cells which survive longer than the normal gestation period. Similarly, blastocysts from the experimental cross show approximately the same survival frequency. The implication from these results is that at least some cells from many of the cultured  $t^{w18}/t^{w18}$  blastocysts are viable well beyond the normal period of lethality of homozygous mutant embryos *in utero* (that is about the 9th day of gestation), a finding consistent with the ectopic implant studies<sup>19</sup>. Inspection of the cultures after three weeks (and at later times) did not reveal the presence of cells in the experimental cross population which were morphologically distinguishable from cells in the control cross population. Several of the single blastocyst-derived cultures from both the experimental and control crosses have been maintained *in vitro* for several months, and experiments are in progress to determine their genotype.

### Conclusions

According to the organisational hypothesis,  $t$  mutations "affect discrete steps in development by impairing the differentiation of particular embryonic cell types"<sup>14</sup>, presumably by interference with "processes necessary to cell-cell interaction and determination"<sup>1</sup>. The experiments described above, as well as others already published<sup>7–11</sup>, are inconsistent with this hypothesis, at least insofar as three classes of early-acting  $t$  mutations ( $t^{12}$ ,  $t^6$ ,  $t^{w5}$ ) are concerned. In each case, all cells of the embryo, rather than specific cell types, are adversely affected. The facts that cells from  $t^6$ -mutant embryos fail to survive in ectopic sites as well as in culture, and that some cells from  $t^{w18}$ -mutant embryos seem to survive under both conditions, reduces the possibility that the observed generalised effect of early-acting  $t$  mutations is due to inadequate culture conditions. Our observations with  $t^6$ -mutant embryos also argue against impairment of all differentiation in affected cells, since  $t^6/t^6$  trophoblast cells, clearly abnormal in that they fail to endoreduplicate like their normal counterparts,

nevertheless, express characteristic differentiation markers (plasma-activator,  $\Delta^5$ , 3  $\beta$ -hydroxysteroid dehydrogenase). Furthermore, the block to endoreduplication is unlikely to be related to improper cell-cell interaction, either self or non-self, since we have found that normal trophectoderm cells are capable of initiating and continuing endoreduplication, even when isolated as single cells (S.B. Atienza and M.I.S., unpublished observations).

It is difficult to place the observed alterations in surface antigens of  $t$ -mutant sperm<sup>3</sup> and embryos<sup>3,17</sup> into perspective with our observations because unequivocal evidence has not been presented to indicate whether such antigens are primary  $t$ -gene products, secondary effects resulting from mutation in the T region, or products of genes closely linked to the T complex which seem to be part of it due to suppression of recombination. Of course, surface alterations can lead to cell lethality in ways other than those involving faulty cell-cell interactions; for example, they might interfere with intracellular processes such as those related to replication and the cell cycle<sup>32</sup>.

We do not yet know why some cells in embryos homozygous for later-acting  $t$  mutations are able to survive and proliferate while all cells in early-acting  $t$ -mutant embryos die. It may be that some cells at advanced stages of differentiation do not express the altered product(s) or require the wild-type product(s) of the T-complex genes. We stress, however, that there is a danger in making comparisons between the various classes of lethal  $t$  mutations until more is learned about the number and nature of genes in the T complex.

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- Bennett, D. *Cell* **6**, 441–454 (1975).
- Sherman, M. I. & Wudl, L. R. in *Concepts in Mammalian Embryogenesis* (ed. Sherman, M. I.) 136–234 (MIT Press, Cambridge, Massachusetts, 1977).
- Artzt, K., Bennett, D. & Jacob, F. *Proc. natn. Acad. Sci. U.S.A.* **71**, 811–814 (1974).
- Bennett, D. in *The Early Development of Mammals* (eds Balls, M. & Wild, A. E.) 201–218 (Cambridge University Press, London, 1975).
- Bennett, D., Boyse, E. A. & Old, L. J. in *Cell Interactions. Lepetit Coll. Biol. Med.* **3** (ed. Silvestri, L.) 247–263 (North-Holland, Amsterdam, 1972).
- Spiegelman, M. in *Embryogenesis in Mammals. Ciba Foundation Symposium* **40**, 199–226 (North-Holland, Amsterdam, 1976).
- Mintz, B. *J. exp. Zool.* **157**, 273–292 (1964).
- Hillman, N., Hillman, R. & Wileman, G. *Am. J. Anat.* **128**, 311–340 (1970).
- Hillman, N. & Hillman, R. *J. Embryol. exp. Morph.* **33**, 685–695 (1975).
- Nadjick, M. & Hillman, N. *J. Embryol. exp. Morph.* **33**, 697–713 (1975).
- Wudl, L. R. & Sherman, M. I. *Cell* **9**, 523–531 (1976).
- Sherman, M. I. *Cell* **5**, 343–349 (1975).
- Sherman, M. I. *Differentiation* **3**, 51–67 (1975).
- Spiegelman, M., Artzt, K. & Bennett, D. *J. Embryol. exp. Morph.* **36**, 373–381 (1976).
- Bennett, D. & Dunn, L. C. *J. Morph.* **103**, 135–158 (1958).
- Dunn, L. C. & Gluecksohn-Schoenheimer *Proc. natn. Acad. Sci. U.S.A.* **36**, 233–237 (1950).
- Bennett, D. & Dunn, L. C. *J. exp. Zool.* **143**, 203–219 (1960).
- Erickson, R. P. & Pedersen, R. A. *J. exp. Zool.* **193**, 377–384 (1975).
- Artzt, K. & Bennett, D. *J. natn. Cancer Inst.* **48**, 141–158 (1972).
- Tarkowski, A. K. & Wroblewska, J. *J. Embryol. exp. Morph.* **18**, 155–180 (1967).
- Sherman, M. I. in *The Early Development of Mammals* (eds Balls, M. & Wild, A. E.) 145–166 (Cambridge University Press, London, 1975).
- Strickland, S., Reich, E. & Sherman, M. I. *Cell* **9**, 231–240 (1976).
- Barlow, P. W. & Sherman, M. I. *J. Embryol. exp. Morph.* **27**, 447–465 (1972).
- Sherman, M. I., Atienza, S. B., Salomon, D. S. & Wudl, L. R. in *Development in Mammals 2* (ed. Johnson, M. H.) 209–233 (North-Holland, Amsterdam, 1977).
- Sherman, M. I., Strickland, S. & Reich, E. *Cancer Res.* **36**, 4208–4216 (1976).
- Chew, N. J. & Sherman, M. I. *Biol. Reprod.* **12**, 351–359 (1975).
- Fawcett, D. W. *Anat. Rec.* **108**, 71–91 (1950).
- Billington, W. D., Graham, C. F. & McLaren, A. *J. Embryol. exp. Morph.* **20**, 391–400 (1968).
- Gardner, R. L. & Johnson, M. H. *J. Embryol. exp. Morph.* **28**, 279–312 (1972).
- Snell, G. D. & Stevens, L. C. *Biology of the Laboratory Mouse 2nd edn* (ed. Green, E. L.) 205–245 (McGraw-Hill, New York, 1966).
- Kemler, R., Babinet, C., Condamine, H., Gachelin, G., Guenet, J. L. & Jacob, F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4080–4084 (1976).
- Edelman, G. M. *J. Biochem.* **79**, 1p–12p (1976).
- Biggers, J. D., Whitten, W. K. & Whittingham, D. G. *Methods in Mammalian Embryology* (ed. Daniel, J. C., Jr) 86–116 (Freeman, San Francisco, 1971).

# articles

## Transformation of the state of nitrogen in diamond

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*High temperature-high pressure annealing experiments on diamond transform type IB dispersed nitrogen into type IA aggregate nitrogen. The activation energy for the transformation is approximately  $60 \text{ kcal mol}^{-1}$  (2.6 eV). An estimate is given for the diffusivity of nitrogen in diamond for this transformation*

NITROGEN impurity is found in substitutional lattice positions in most natural and synthesised diamonds, but its usual state differs in the two types of diamond. Most natural diamonds that contain nitrogen have it in a form that is inactive to electron paramagnetic resonance (EPR) and these diamonds have been classified type IA. In the past this form of nitrogen has been pictured as localised in the platelets characteristic of type IA crystals, but evidence suggests the nitrogen may occur in clusters of 10–13 atoms<sup>1</sup> or substitutional nitrogen pairs<sup>2</sup> rather than in platelets. Nitrogen in synthesised diamonds is EPR active and atomically dispersed. These diamonds have been placed in the type IB classification.

A few natural diamonds are type IB or a mixture of types IA and IB. No synthesised diamonds with wholly type IA properties have been made, but some type IA characteristics in certain synthesised diamonds have been reported<sup>3,4</sup>.

The existence of the two diamond types poses questions about their relative stability and whether the stable type may be formed by a high temperature-high pressure treatment in the laboratory. If successful, this might provide an insight into the history of natural diamonds and give important information for understanding their growth mechanisms. For example, Klyuev *et al.*<sup>3</sup> and Evans<sup>5</sup> suggested that nitrogen deposits in the atomically dispersed state (type IB) in natural diamond during growth, with the type IA state forming during the long residence in the high temperature environment deep within the earth. Is this hypothesis consistent with experimental data and could approximate residence times be ascertained?

Our experiments have indicated high temperature-high pressure treatment transforms type IB nitrogen into type IA nitrogen. This has provided information about the reaction rates for the transformation IB to IA and the diffusivity of nitrogen in diamond. Some implications are drawn from the data about the growth of natural diamonds. More experiments should clarify some old questions about platelets, X-ray spikes, and gem manufacturing.

### Experimental methods

The difficult experimental conditions necessary for transforming nitrogen states in diamond are dictated by the extraordinary refractory nature of diamond as shown by its high melting point and resistance to plastic flow. The melting point is about 4,000 K at 150 kbar (ref. 6) and no plastic yielding occurs below about 2,073 K (ref. 7). Hence, any process requiring lattice diffusion of nitrogen could not be expected to proceed rapidly below temperatures of about 2,000 K. These experiments were conducted at this temperature level under pressures in the range 55–65 kbar to prevent graphitisation of the diamonds.

The GE 'Belt' pressure apparatus<sup>8</sup> was used to provide the

pressurised environment for the diamond heat treating experiments. The crystals to be heated, 1–2 mm in size, were placed in a small cavity in the centre of a 3.17 mm diameter graphite rod (Fig. 1). The temperatures reached in terms of power input were measured several times with a 0.127 mm Pt–Pt 10% Rh thermocouple. Temperatures in terms of power input were consistent to within about  $\pm 75^\circ$  at the 2,273 K level. Part of the variation from one test to another was due to the differing cross sections of the crystals used. This variation influenced the heating current density in the graphite rod at the diamond where its cross section was reduced by different amounts due to the presence of diamond of different sizes.

Crystals were treated at temperatures between 1,873 K and 2,273 K, usually for 30 min at a time, at pressures near to or within the region for the thermodynamic stability of diamond at the treatment temperature, 55–65 kbar.

The analytical techniques used to monitor the conversion of the nitrogen from the IB to IA state were dictated by the characteristic properties of each state. Type IA and type IB nitrogen each induce their own characteristic ultraviolet, visible, and infrared absorptions; type IA nitrogen is apparently EPR inactive but type IB nitrogen is EPR active. Many type IA diamonds are colourless whereas most type IB crystals are canary yellow.

Quantitative measurements of types IA and IB nitrogen concentrations depend on the infrared absorption intensities of bands at  $1,280 \text{ cm}^{-1}$  for the type IA nitrogen and at  $1,130 \text{ cm}^{-1}$  for type IB nitrogen. These two band systems have been closely correlated with nitrogen content by Kaiser and Bond<sup>9</sup> for type IA nitrogen and Chrenko, Strong and Tuft<sup>10</sup> for type IB nitrogen. Absorption spectra recorded for each crystal before and after heat treatment provided information on the amounts of each type of nitrogen present and the total in the crystal in these two states. Any change in this nitrogen total would indicate either a change in the amount of nitrogen in the crystal or a transformation to some other state not yet identified.

EPR was used in a semi-quantitative manner. The decrease in integrated intensity (amplitude  $\times$  width<sup>2</sup>) of the centre line of the dispersed nitrogen hyperfine triplet was a measure of the decrease

Fig. 1 Sample holder for diamond used in the high pressure apparatus. The alumina liner protected the pyrophyllite sample holder from the high core temperature. The thermocouple was used in several experiments for calibration of temperature in terms of power input. For most annealing experiments the thermocouple was left out.

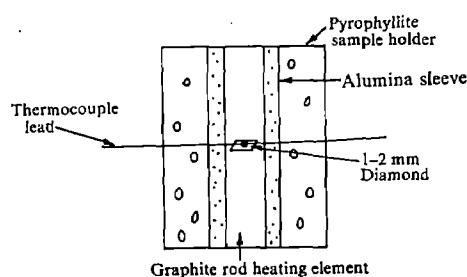


Table 1 EPR, infrared, and ultraviolet-visible data

Crystal*	T (K)	Total time at T (min)	EPR		Infrared	Type IA nitrogen§ (p.p.m.)	IA and IB nitrogen (p.p.m.)	Ultraviolet-Visible 415 nm Absorption line
			F†	Type IB nitrogen‡ (p.p.m.)				% Transmission at 400 nm
N-1	—	—	1.00	96	1.00	37	133	~3
S-1	2,173	30	0.38	42	0.44	93	135	~7
	—	—	1.00	365	1.00	0	365	~0
	2,173	30	0.32	128	0.35	234	362	~5
S-2	2,173	60	0.15	74	0.20	288	362	~5.3
	—	—	1.00	237	1.00	0	237	~0
	1,973	30	0.79	180	0.76	24	204	~0

\*N, natural diamond; S, synthesised diamond; N-1 was a mixed IA-IB crystal originally

†F, amount of type IB nitrogen remaining/original amount of type IB nitrogen

‡From 1,343 cm<sup>-1</sup> absorption; see text.

§From 1,280 cm<sup>-1</sup> absorption.

in dispersed nitrogen content. The intensities were normalised to unit intensity for the untreated crystal.

The ultraviolet and visible absorptions were not very useful for quantitative measurements because the absorption occurs in a poorly defined absorption tail. The increased transmission in the 350–500 nm region and emergence of the 415.2 nm zero phonon line of the N-3 absorption system, however, were characteristic of the transformation from type IB to IA diamond. These latter measurements therefore, confirmed that the type IB to type IA transformation was occurring.

## Results

After heat treatment, the deep yellow colour of type IB crystals had faded significantly. Quantitative measurements on their spectral absorption and EPR characteristics yielded data on the transformation from IB to IA that are summarised in Table 1.

Infrared absorption spectra for a synthesised type IB crystal, before and after heat treatment at 2,173 K for 30 min, are shown in Fig. 2. A natural type IA crystal absorption spectrum is included for comparison. The partial conversion of the type IB crystal to type IA is evident.

The quantitative measurements from infrared absorption data showed that concentrations of type IB nitrogen decreased while the concentrations of type IA nitrogen correspondingly increased. The total nitrogen remained constant. Measurements of type IB nitrogen were actually made using the absorption at 1,343 cm<sup>-1</sup>, which is correlated with the 1,130 cm<sup>-1</sup> absorption, since the 1,130 cm<sup>-1</sup> absorption becomes difficult to resolve as it weakens while the 1,343 cm<sup>-1</sup> absorption remains sharp and distinctive. Type IA nitrogen concentrations were obtained from its 1,280 cm<sup>-1</sup> absorption. The EPR data showed close agreement with infrared absorption data on decreases in concentrations of type IB nitrogen. The ultraviolet-visible data indicated increased transmission in the 350–500 nm region with the transformation IB to IA nitrogen, in agreement with the quantitative measurements on the transformation. In the samples wherein sufficient conversion had taken place, the 415 nm zero phonon line of the N-3 system of type IA diamonds appeared, confirming the previous evidence on the nature of the transformation.

Heat treatment of type IB synthesised crystals at 2,273 K produced only the type IA group A bands characterised by the absorption at 1,280 cm<sup>-1</sup>. No evidence was seen for the group B bands, whose strongest absorption is at 1,175 cm<sup>-1</sup>. Also, heat treatment at 2,273 K of a natural diamond having both the 1,280 cm<sup>-1</sup> and 1,175 cm<sup>-1</sup> group A and group B absorptions produced no shift in relative intensities of either band.

Quantitative measurements on the extent of the reaction in terms of time and temperature indicated that the transformation was controlled by second order diffusion kinetics obeying the rate equation:

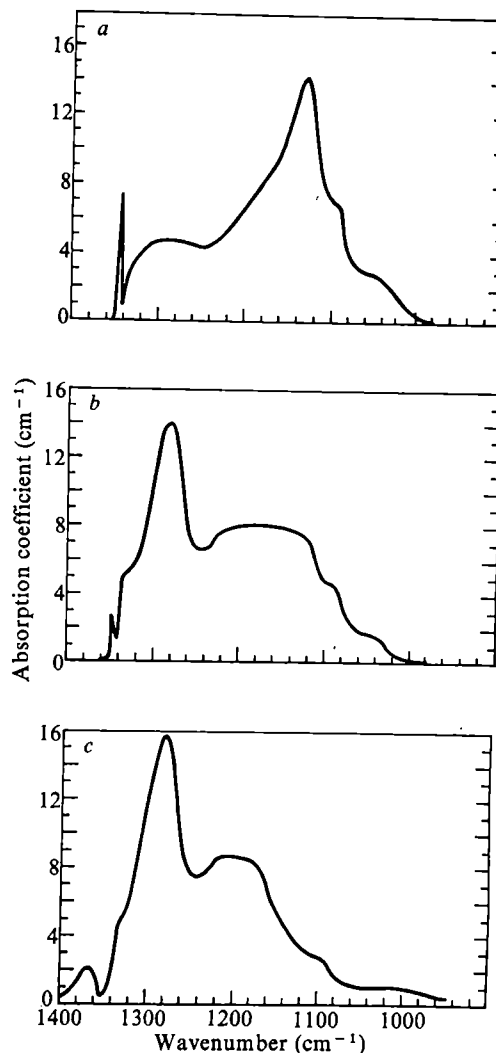
$$kt = 1/c - 1/c_0$$

in which  $k$ , the rate constant, was expressed as min<sup>-1</sup> p.p.m.<sup>-1</sup>;  $t$  is the time in min;  $c_0$  the initial concentration of type IB nitrogen in

p.p.m. and  $c$  the concentration of type IB nitrogen remaining after heat treatment. In Fig. 3,  $1/c$  is plotted against  $t$  for the data at 2,173 K which gives  $k = 1.74 \times 10^{-4}$  min<sup>-1</sup> p.p.m.<sup>-1</sup>.

An Arrhenius plot for all of the reaction rate data is shown in Fig. 4. The scatter in the data points may be attributed in part to uncertainties in temperature measurement and to the fact that nitrogen can be unevenly distributed within diamond crystals. During growth, nitrogen has a strong tendency to collect in streaks

Fig. 2 Infrared absorption spectra for *a*, a type IB synthesised diamond as grown; *b*, the same diamond after a heat treatment for 30 min under pressure at 2,173 K; *c*, a typical type IA natural diamond, for comparison purposes.



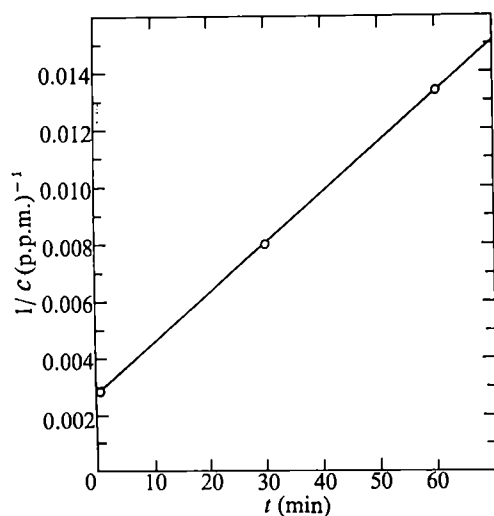


Fig. 3 A plot of  $1/c$  against time in min for a type IB diamond run at 2,173 K.  $c$  is the concentration of type IB nitrogen in p.p.m. A value of  $k = 1.74 \times 10^{-4} \text{ min}^{-1} \text{ p.p.m.}^{-1}$  is obtained from this data.

and patches along certain crystal planes instead of distributing itself uniformly. The infrared and EPR measurements do not differentiate between concentrated and evenly distributed nitrogen. They measure only the total dispersed nitrogen present. Because the apparent reaction rate is proportional to the concentration of dispersed nitrogen the reaction in a concentrated nitrogen streak would be seen as running much faster than it would if the nitrogen were more evenly dispersed.

The reaction rate may also be influenced by the presence of dislocations. Conceivably, certain types of dislocations would facilitate the diffusion of nitrogen in diamond thereby speeding up the formation of type IA nitrogen.

From the Arrhenius plot in Fig. 4, the activation energy for rate constant was found to lie within the range 48 to 73 kcal mol<sup>-1</sup>. Using the equation  $k = A \exp(-E/RT)$ , assuming an activation energy for the reaction rate of 60 kcal mol<sup>-1</sup> (2.6 eV) and a  $k$  value of  $1.74 \times 10^{-4} \text{ min}^{-1} \text{ p.p.m.}^{-1}$  at 2,173 K, a set of  $k$  values for the temperature range 1,573 to 2,273 K was obtained. A table of times for the reaction to reach 50, 99.0, and 99.9% completion at temperatures between 1,573 and 2,273 K was then prepared (Table 2) for an assumed initial concentration of type IB nitrogen of  $c_0 = 500 \text{ p.p.m.}$

These experiments provide an opportunity to obtain some rough estimates on the diffusivity of nitrogen in diamond for the type IB to type IA reaction. This was done by assuming type IA nitrogen to be in the form of clusters and making certain other assumptions about the size and number of clusters formed, as well as the concentration gradient of dispersed type IB nitrogen between clusters.

The calculation for the diffusivity is based on Fick's first law,  $\Delta N = D(dc/dx)At$ ; in which  $\Delta N$  is the number of atoms of

nitrogen in a 1 cm<sup>3</sup> diamond which are collected into clusters,  $D$  is the diffusivity in cm<sup>2</sup> s<sup>-1</sup>,  $dc/dx$  is the concentration gradient expressed as number of atoms  $\times \text{cm}^{-4}$ ,  $A$  the effective diffusion area in cm<sup>2</sup>, and  $t$  the time in s.

The effective diffusion area,  $A$ , was taken as the area of one 13 atom cluster multiplied by the average number of clusters present during the initial 30-min anneal. One cluster was estimated to have the area of a  $4 \times 10^{-8} \text{ cm}$  cube. The example used for calculating  $D$  was taken from Table 1 in which a crystal (S-1) having 365 p.p.m. type IB nitrogen originally was heated for 30 min at 2,173 K and then found to have its type IB nitrogen reduced to 128 p.p.m. Hence, in a 1 cm<sup>3</sup> crystal the number of cluster nitrogen atoms,  $\Delta N$ , would have been  $42 \times 10^{18}$ . The average number of clusters was  $10^{18}$  and the cluster spacing was  $10^{-6} \text{ cm}$ .  $dc/dx$  was assumed to be  $42 \times 10^{18} \text{ cm}^{-3}$  divided by 1/2 the spacing between clusters or  $5 \times 10^{-7} \text{ cm}$ . With these assumptions, the value of  $D$  at 2,173 K is  $\sim 3 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$ .

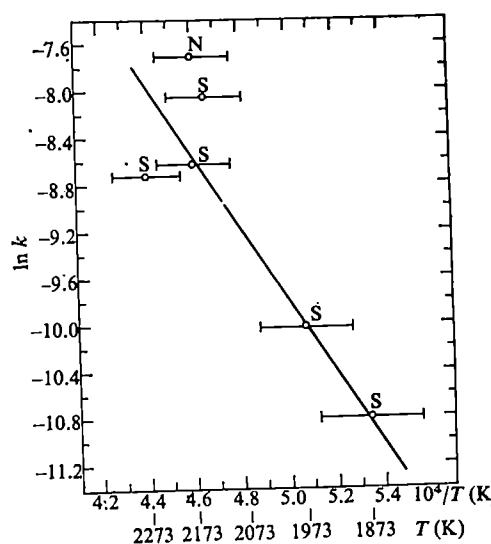


Fig. 4 A plot of  $\ln k$  values against  $10^4/T$  for all the experiments. The slope of the drawn line corresponds to an activation energy of 60 kcal mol<sup>-1</sup>. S, synthesised diamond; N, natural diamond.

This value of the diffusivity of nitrogen in diamond may be compared to the diffusivity of carbon in diamond from data supplied by Swalin<sup>11</sup>. Swalin gave values for  $D_0$  of 11.6 cm<sup>2</sup> s<sup>-1</sup> and  $Q$  of 143 kcal mol<sup>-1</sup> in

$$D = D_0 \exp(-Q/RT)$$

At 2,173 K this gives  $D = 6 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$ . Hence, in consideration of the nearly equal sizes of the nitrogen and carbon atoms, a reasonable value for the diffusivity of nitrogen for the type IB to type IA transformation in diamond should be in the order of  $10^{-14} \text{ cm}^2 \text{ s}^{-1}$  at 2,173 K provided that the cluster model for aggregate type IA diamond is correct.

Table 2 Reaction rates at different temperatures and times required to reach 50, 99 and 99.9 conversion of type IB to type IA nitrogen

T (K)	k (min <sup>-1</sup> p.p.m. <sup>-1</sup> )	c <sub>0</sub> (p.p.m.)	t <sub>50%</sub> = 1/c <sub>0</sub> k (min)	Conversion times	
				t <sub>99%</sub> = 99/c <sub>0</sub> k	t <sub>99.9%</sub> = 999/c <sub>0</sub> k
2,273	3.2 × 10 <sup>-4</sup>	500	6.3	10 h	4.3 d
2,173	1.74 × 10 <sup>-4</sup>	500	11	19 h	8.0 d
2,073	8.9 × 10 <sup>-5</sup>	500	22	37 h	16 d
1,973	4.3 × 10 <sup>-5</sup>	500	47	77 h	32 d
1,873	1.9 × 10 <sup>-5</sup>	500	105	175 h	73 d
1,773	7.7 × 10 <sup>-6</sup>	500	260	18 d	0.5 yr
1,673	2.8 × 10 <sup>-6</sup>	500	715	49 d	1.4 yr
1,573	9.0 × 10 <sup>-7</sup>	500	2,200	150 d	4.2 yr

Initial concentration,  $c_0$ , assumed to be 500 p.p.m. type IB nitrogen. Calculations based on:  $k = A \exp(-E/RT)$  with  $E = 60 \text{ kcal mol}^{-1}$  and  $k = 1.74 \times 10^{-4} \text{ min}^{-1} \text{ p.p.m.}^{-1}$  at 2,173 K.



## Conclusions and discussion

At temperatures between 1,873 K and 2,273 K the type IA nitrogen state in diamond is the stable form, not the type IB state. In this temperature region the diamond states responsible for both group A and group B type IA absorption bands seem to be stable. The experiments also suggest that the transformation of dispersed type IB nitrogen into type IA nitrogen is more favourable kinetically than diffusion of dispersed nitrogen out of the diamond.

The activation energy for the type IB to type IA nitrogen transformation is approximately  $60 \text{ kcal mol}^{-1}$  (2.6 eV) over the temperature range 1,873 K to 2,273 K. Assuming a cluster model for aggregate type IA nitrogen, an approximate value for the diffusivity of nitrogen for the type IB to type IA transformation should be in the order of  $10^{-14} \text{ cm}^2 \text{ s}^{-1}$  at 2,173 K.

Continuing studies should give some insight into some old questions about diamonds. For example, it was believed that the platelets seen in type IA diamonds with the transmission electron microscope were composed of a nitrogen-carbon complex. New evidence suggests the platelets are composed of interstitial carbon<sup>5,12</sup>, with nitrogen apparently necessary for the formation of the platelets but not being incorporated into them in any appreciable amount. Transmission electron microscope studies on the present transformed type IB synthesised diamonds should help resolve the role of nitrogen in the formation of platelets. A parallel study on X-ray diffraction spikes should confirm any findings about platelets.

It has been considered that the various optical features of type IA diamond such as the  $1,280 \text{ cm}^{-1}$  absorption band or the N-3 absorption and emission system with its sharp zero phonon line were found only in natural diamonds. Hence, the finding of the N-3 emission system in the Hannay diamonds was taken as proof for their natural origin<sup>13</sup>. This assumption is probably correct since these features are related to aggregate nitrogen and the required annealing conditions for transforming the type IB dispersed nitrogen found in synthesised diamonds into type IA aggregate nitrogen have not been attainable until recently. These annealing conditions were almost certainly not attainable in the laboratory during Hannay's time. Present experiments have shown what these conditions are: 1,673 K at 60 kbar for ~50 d or 1,873 K at 60 kbar for ~7 d for 99% conversion of type IB to type IA nitrogen (see

Table 2). These times and temperatures are appreciably greater than those reported by Hannay<sup>14</sup>. More detailed experiments will be needed to differentiate between natural and synthesised diamonds in the future.

The colouration change that occurs on annealing type IB crystals suggests that large, yellow type IB synthesised diamonds<sup>15</sup> that are grown slowly to obtain gem quality crystals could be turned into colourless crystals by proper annealing.

It now seems quite certain that during the growth of diamond in a nitrogen containing environment, whether naturally or in the laboratory, nitrogen deposits substitutionally in diamond in an atomically dispersed state. With prolonged dwell times in the growth environment, the nitrogen state changes, quite probably forming aggregates which impart characteristic type IA properties.

The time for completion of the reaction is rather short on a geological time scale. The fact that some natural diamonds do contain a substantial amount of atomically dispersed nitrogen suggests that these crystals were exposed to temperatures above 1,300 to 1,500 K for only relatively short times during growth and later transport to the surface through the internal churnings of the mantle.

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1. Berman, R., Hudson, P. R. W. & Martinez, M. *J. Phys. C, Solid St. Phys.* **8**, L430-L434 (1975).
2. Davies, G. *J. Phys. C: Solid St. Phys.* **9**, L537-L542 (1976).
3. Klyuev, Yu. A., Hepsha, V. I. & Naletov, A. M. *Sov. Phys. Solid St.* **16**, 2118-2121 (1975).
4. Klimentova, N. T., Prokopchuk, E. O., Laptev, V. A. & Roiter, Yu. M. *Opt. Spectrosc.* **39**, 189-190 (1975).
5. Evans, T. *Contemp. Phys.* **17**, 45-70 (1976).
6. Bundy, F. P. *Science* **137**, 1057-1058 (1962).
7. Evans, T. & Wild, R. K. *Phil. Mag.* **12**, 479-489 (1965).
8. Hall, H. T. *Rev. Scient. Instrum.* **31**, 125-131 (1960).
9. Kaiser, W. & Bond, W. L. *Phys. Rev.* **115**, 857-863 (1959).
10. Chrenko, R. M., Strong, H. M. & Tuft, R. E. *Phil. Mag.* **23**, 313-318 (1971).
11. Swalin, R. A. *J. Phys. Chem. Solids* **18**, 290-296 (1961).
12. Woods, G. S. *Phil. Mag.* **34**, 993-1012 (1976).
13. Collins, A. T. *Ind. Diam. Rev.* 434-437 (1975).
14. Hannay, J. B. *Nature* **22**, 255-257 (1880).
15. Strong, H. M. & Chrenko, R. M. *J. phys. Chem.* **75**, 1838-1843 (1971).

# Terrestrial lead isotopic evolution and formation time of the Earth's core

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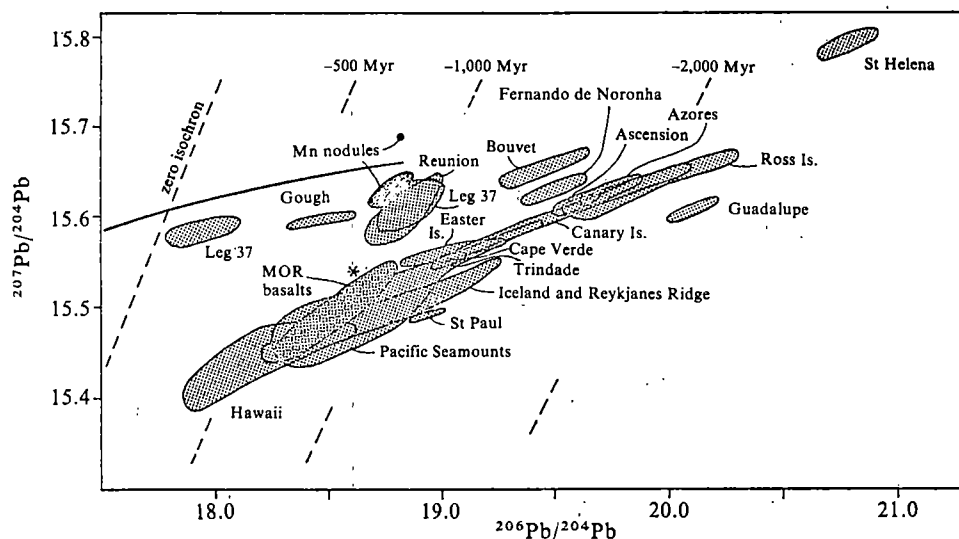
*The radiogenic nature of Pb in average crust and mantle probably requires some interaction between mantle and core during core formation and is thus incompatible with heterogeneous accretion models for the Earth. Pb isotope evolution models for hot and cool accretion indicate that core formation was not quasi-simultaneous with accretion although the core was probably 95% completed within the first 700 Myr of the Earth's history.*

THE high precision Pb isotope data which has become available in the past few years enables average Pb isotopic composition for the sampled mantle and crust (Fig. 1) to be estimated. The mid-oceanic-ridge (MOR) basalts occupy a restricted field, whereas oceanic islands extend linearly to more radiogenic Pb. The most reliable estimate of average crustal Pb is probably given by the present-day ratios of the conformable Pb ore growth curve<sup>1</sup> which represents the Pb isotopic evolution of average crustal Pb<sup>2,3</sup>. Another estimate is obtained from Pb in manganese nodules and

oceanic sediments. This Pb has its origin in continental waters, as seawater has an order of magnitude lower Pb concentrations, and as the mean resident time of Pb in seawater is short (~3,500 yr)<sup>4</sup> it will give the average Pb of the eroded continental surface. There is a good agreement between both estimates (Fig. 1). It is evident from the difference in the <sup>207</sup>Pb/<sup>204</sup>Pb ratio that continental crust, at least in the past  $1 \times 10^9$  yr but possibly from early on in the Earth's history, has followed a different Pb isotopic evolution than oceanic mantle. This precludes a steady growth of the continental crust until the present.

If the crust-mantle U concentration is estimated to be 0.035 p.p.m.<sup>5</sup>, then a  $\mu$  value (<sup>238</sup>U/<sup>204</sup>Pb) of 9.5 will yield 0.0037 p.p.m. <sup>204</sup>Pb. From continental crustal abundances for U of 1.9 p.p.m. (ref. 6) and for Pb of 12.5 p.p.m. (ref. 7), a mass balance calculation gives 32% of U and 27% of Pb in the crust now. Despite uncertainties of up to 50% in some estimates a crustal  $\mu$  value of 11 derived from these figures is in excellent agreement with a present-day  $\mu$  value of 10.75 resulting from the Pb ore growth curve<sup>1</sup>. Although Pb and U are both strongly enriched in the crust, U is probably only slightly enriched relative to Pb. A similar geochemi-

**Fig. 1** Lead isotopic composition of oceanic basalts and manganese nodules. Solid line, conformable lead ore growth curve; \*, estimate of average sampled crust-mantle Pb isotopic composition. Source of data: MOR basalts (ref. 9); oceanic islands (refs 9, 21-25); manganese nodules (refs 26, 27 and R.V. unpublished); conformable Pb ore growth curve (ref. 1); reference isochrons (refs 10, 17).



cal behaviour of U and Pb is demonstrated by only considering oceanic Pb: MOR basalts seem to be derived from a previously depleted source region. They contain low concentrations of LIL (large ion lithophile) elements and contain also less radiogenic Pb (and Sr) than oceanic islands basalts<sup>8,9</sup> (Fig. 1). Enrichment in LIL elements is again correlated with a slight increase in the  $\mu$  value. Because of this geochemical behaviour the best estimate for average oceanic Pb is probably not the average MOR basalt but a point shifted somewhat to the more radiogenic side within the MOR basalt field. From these figures the average Pb isotopic composition for the sampled crust-mantle system is estimated to be  $^{206}\text{Pb}/^{204}\text{Pb} \approx 18.6$  and  $^{207}\text{Pb}/^{204}\text{Pb} \approx 15.54$ .

Comparison with meteoritic Pb (Fig. 1) reveals that the sampled mantle and crust is radiogenic in excess of a single-stage evolution (an evolution with constant U/Pb ratios) dating from  $4.57 \times 10^9$  yr, when the Pb in the Earth had the same isotopic composition as the Pb in meteorites; this time is commonly regarded as the time of accretion of the Earth. This radiogenic nature is particularly pronounced when the revised U decay constants of Jaffey *et al.*<sup>10</sup> are used, and is demonstrated by large negative single-stage model ages from -600 Myr to -1,000 Myr for MOR basalts and ~ -800 Myr for the estimated average sampled crust-mantle system (Fig. 1). This cannot be attributed to uncertainties in the estimated crustal Pb. For the average crust-mantle system to fall on the meteoritic zero isochron, the average crust would have to be extremely unradiogenic ( $^{206}\text{Pb}/^{204}\text{Pb} \sim 14-15$ , corresponding to a model age of ~ 2,500 myr). This would be far beyond the limits of uncertainty in this estimate, and contrary to the observation that U and Pb are on the whole very little fractionated. As it is unlikely that the Earth is substantially younger than meteorite parent bodies (a single-stage evolution, under the condition of a large U-Pb fractionation during accretion, would yield less than 4,420 Myr), it follows, therefore, that the average Pb of the sampled crust-mantle system is almost certainly radiogenic in excess of a closed system evolution. U must have been added and/or Pb been removed from this system sometime during its history since the accretion of the Earth.

It has been postulated that the unsampled (presumably lower) mantle is the source of the excess U. For example, Church and Tatsumoto<sup>9</sup> suggest that mantle convection extends to depth exceeding the pyroxene-garnet transformation, resulting in a strong exclusion of large ion lithophile (LIL) elements from the mantle mineralogy and in their concentration in an interstitial phase. Separation of this interstitial phase would leave the lower mantle depleted and the upper mantle enriched in LIL elements, allowing  $\mu$  to increase with time in the upper mantle. This model, however, is incompatible with a chondritic rare earth abundance pattern for the bulk crust-mantle system. As the source of MOR basalts seems to be depleted in LIL elements and thus in light rare earth elements and has been so for much of the Earth's history<sup>11-13</sup>, the model proposed by Church and Tatsumoto would demand that the lower mantle, and thus the bulk crust-mantle

system be even more strongly depleted in light REE than the source of MOR basalts. This is extremely unlikely.

An obvious, and possibly the only conceivable way a radiogenic crust-mantle system may be generated would be by enrichment in U and depletion in Pb during core formation. This requires some interaction between mantle and core during core formation (but does not necessarily entail equilibrium between core and mantle) and is thus incompatible with, and a strong argument against, a heterogeneous accretion model for the Earth.

In the following, two simple models of core formation based on Pb isotope data are presented. Model 1 is identical with the model of Oversby and Ringwood<sup>14</sup> who start from the same basic premise; however, the revised U decay constants of Jaffey *et al.*<sup>10</sup> will significantly alter the results. In both models it is assumed that all U present in the undifferentiated Earth is accumulated in the mantle and none is retained in the core. This seems to be a reasonable assumption from our knowledge of the chemistry of the core<sup>15</sup>. The Pb partition coefficient between a silicate melt and iron-nickel with minor amounts of sulphide or silicate has been measured by Oversby and Ringwood<sup>14</sup>. Their best estimate for the core-mantle Pb partition coefficient is  $D_{\text{Pb}}^{\text{c/m}} = 2.5 \pm 0.5$  although some uncertainty exists because of the required large extrapolation to high pressures.

## Model 1

The specific boundary condition (in addition to those outlined above) for this model is: the time of core formation is assumed to be short in comparison to that required to change significantly the Pb isotopic composition by decay, so that core formation may be regarded as a discrete and instantaneous process. This approximation is valid if core formation occurred within a few Myr. This will reduce the Pb isotopic evolution to a simple two-stage evolution model with constant  $\mu$  in the first stage, for which is valid<sup>16</sup>:

$$\frac{\mu^{\text{Earth}}}{\mu^{\text{m}}} = \frac{A^{\text{m}} (\exp \lambda' t_c - 1) - B^{\text{m}} (\exp \lambda t_c - 1)}{B^{\text{m}} (\exp \lambda t_p - \exp \lambda t_c) - A^{\text{m}} (\exp \lambda' t_p - \exp \lambda' t_c)} \quad (1)$$

where  $A^{\text{m}} = (^{206}\text{Pb}/^{204}\text{Pb})_0^{\text{m}} - a_p$ ,  $B^{\text{m}} = [(^{207}\text{Pb}/^{204}\text{Pb})_0^{\text{m}} - b_p] / 137.88$ ,  $\lambda$  and  $\lambda'$  are the  $^{235}\text{U}$  and  $^{238}\text{U}$  decay constants, and  $\mu$  is the  $^{238}\text{U}/^{204}\text{Pb}$  ratio normalised for U decay to its present-day value; m and c stand for the average crust-mantle and core, respectively, and  $a_p$ ,  $b_p$  are the Pb isotope ratios at  $t_p$  (the time of accretion of the Earth) derived from meteorite Pb<sup>17</sup>. A relation between the  $\mu$  value for the bulk Earth in the first stage from  $t_p$  till  $t_c$  ( $\mu^{\text{Earth}}$ ) and the average  $\mu$  value for the crust-mantle system ( $\mu^{\text{m}}$ ) in the second stage from  $t_c$  till  $t_0$  (the present) is derived through the crust-mantle U and Pb concentrations  $C_{238\text{U}}$ ,  $C_{204\text{Pb}}$  and partition coefficients  $D_{\text{U}}^{\text{c/m}}$  and  $D_{\text{Pb}}^{\text{c/m}}$ .

$$C_{204\text{Pb}_0}^{\text{Earth}} = C_{204\text{Pb}_0}^{\text{m}} [\beta_0 D_{\text{Pb}}^{\text{c/m}} + (1 - \beta_0)] \quad (2)$$

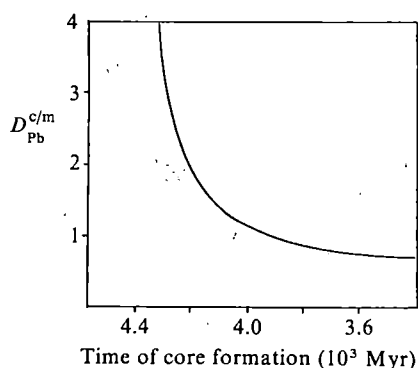


Fig. 2 Model 1: time of core formation as a function of the core-mantle Pb distribution coefficient.

and because  $D_U^{c/m} = 0$

$$C_{238U}^{Earth} = C_{238U}^m (1 - \beta_0) \quad (3)$$

where  $\beta_0$  is the relative mass of the core at the present ( $\beta_0 = 0.32$ ). Equations (2) and (3) combine to

$$\mu^{Earth} = \frac{\mu^m (1 - \beta_0)}{\beta_0 D_{Pb}^{c/m} + 1 - \beta_0} \quad (4)$$

The results are plotted in Fig. 2. The sum of all uncertainties involved (except uncertainty in the distribution coefficient) may be approximated to be variations in the single-stage model age. The revised U decay constants of Jaffey *et al.*<sup>10</sup> causes a change in the model age by a factor of 2 to 3 and, therefore, the results are significantly different from those obtained by Oversby and Ringwood<sup>14</sup>, who concluded that the time interval between accretion and formation of the core was probably less than 100 Myr and because of such a short interval, accretion must have resulted in a hot initial Earth. Assuming that the U-decay constants (which are the most important source of any uncertainty) will not significantly change with any further revision, core formation according to this model cannot have occurred before 200 Myr and probably not later than 500 Myr after accretion. Values for  $\mu^{Earth}$  are strongly dependent on the assumed Pb partition coefficient: they are about 4.0, 4.8, and 6.5 for  $D_{Pb}^{c/m}$  equal to 3, 2, and 1, respectively. The average  $\mu$ -value for crust-mantle is about 9.4. Contrary to the interpretation given by Oversby and Ringwood<sup>14</sup>, this model is only compatible with an initially solid Earth being heated by radioactive decay until the metallic phase suddenly segregated into a core.

## Model 2

If core formation is regarded as a first-order rate process, the rate of growth of the core is given by

$$\frac{d\beta}{d\tau} = -\frac{d\beta^*}{d\tau} = \varepsilon\beta^* \quad (5)$$

where  $\beta^*$  is fractional mass of core material, which is present at any time in the mantle either as metallic particles, droplets, or in solution, and is equilibrated with the surrounding silicate phase;  $\varepsilon$  is the growth constant for the core. The mass of core at time  $\tau$  is then

$$\beta_\tau = \beta^* (\exp \varepsilon \tau - 1) \quad (6)$$

if it is assumed that at the time of accretion of the Earth the mass of core had been zero. Equation (6) is written in the commonly used geological timescale ( $t$ ) where  $t_0$  is zero and time is counted positive into the past:

$$\beta_t = \beta_0^* (\exp \varepsilon t_p - \exp \varepsilon t) \quad (7)$$

From equation (7) follows the mass of core at time  $t$  in relation to its present mass:

$$\beta_t = \frac{\beta_0 (\exp \varepsilon t_p - \exp \varepsilon t)}{\exp \varepsilon t_p - 1} \quad (8)$$

The concentration  $C_N$  of a nuclide  $N$  in the residual mantle after the  $n$ -th batch of core material,  $\Delta\beta$ , has segregated from it is related to the concentration of the nuclide in the mantle (relative mass:  $1 - \beta$ ) immediately before this event by

$$C_N^m (1 - \beta)_{n-1} = [\Delta\beta D_N^{c/m} + (1 - \beta)_n] C_{Nn}^m \quad (9)$$

where  $D_N^{c/m}$  is the core-mantle distribution coefficient for the nuclide  $N$ . The change in concentration during this event is given by  $\Delta C_N = C_{Nn} - C_{N(n-1)}$  and the change in the relative mass ( $1 - \beta$ ) of the mantle by  $\Delta\beta = (1 - \beta)_{n-1} - (1 - \beta)_n$ . The concentration at time  $t$  is therefore related to the mass of core at time  $t$  by

$$\int_{C_{Ntp}^m}^{C_{Nt}^m} \frac{-d C_N^m}{C_N^m} = (D_N^{c/m} - 1) \int_0^{\beta_t} \frac{d\beta}{1 - \beta} \quad (10)$$

which integrates to

$$C_{Nt}^m = C_{Ntp}^m (1 - \beta_t)^{D_N^{c/m} - 1} \quad (11)$$

The  $\mu$  value of the mantle at  $t$  will be (approximation  $D_{Pb}^{c/m} - C_{Pb}^c/C_{Pb}^m \approx C_{204Pb}^c/C_{204Pb}^m$ )

$$\mu_t^m = \mu_{tp}^m (1 - \beta_t)^{-D_{Pb}^{c/m}} \quad (12)$$

Finally, the growth of radiogenic  $^{206}Pb$  since  $t_p$  is given by

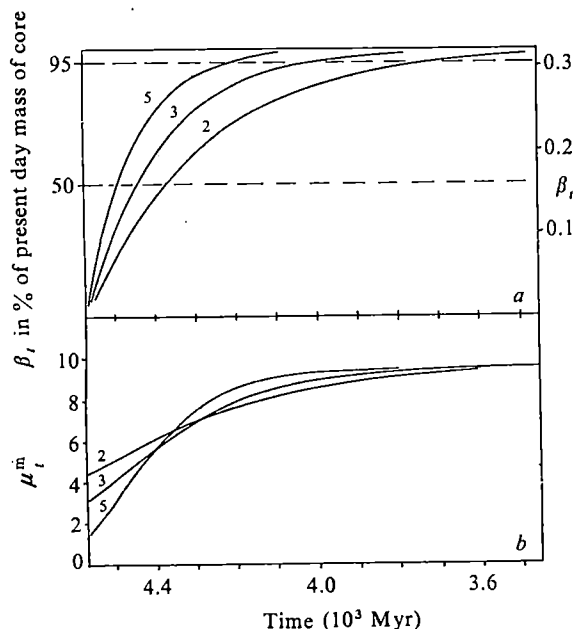
$$\left( \frac{^{206}Pb}{^{204}Pb} \right) - a_p = \int_0^{t_p} \lambda \mu_t^m \exp \lambda t \, dt \quad (13)$$

where  $\mu_t^m$  is an exponential function of time as described by equations (8) and (12). A similar equation may be written for the  $^{235}U$ - $^{207}Pb$  decay system.

The integrals for the  $^{206}Pb/^{204}Pb$  and  $^{207}Pb/^{204}Pb$  ratios were solved numerically. Values obtained for the core growth constant  $\varepsilon$  for core-mantle Pb partition coefficients of 5, 3, and 2 are 8.5, 5.4, and  $3.5 \times 10^{-9} \text{ yr}^{-1}$ , respectively. For a partition coefficient value as low as 1,  $\varepsilon$  cannot be computed. Figure 3a shows the growth of the core with time for the three partition coefficient values and in Fig. 3b is plotted the growth of  $\mu_t^m$  for the same values. The calculated present-day  $\mu$  value for average crust-mantle is about 9.5 and is almost independent of the distribution coefficient and growth constant. The  $\mu$  value for the bulk Earth is, however, above all determined by the Pb distribution coefficient ( $\mu^{Earth} = 1.38 \pm 0.05$ ,  $3.0 \pm 0.1$ ,  $4.4 \pm 0.1$  for  $D_{Pb}^{c/m} = 5, 3, 2$ , respectively). This model is compatible with hot accretion which left the Earth completely or partially molten and enabled metallic droplets to sink towards the centre and form a continuously growing core.

## Discussion and conclusions

Pb isotope data are probably only compatible with homogeneous accretion of the Earth. Simple models corresponding to the two extreme homogeneous accretion hypotheses of (1) relatively cool accretion with subsequent heating by radioactive decay and (2) hot accretion, show that core formation was probably not quasi-simultaneous with accretion, as is widely held, but occurred after or during a period of several hundred Myr following accretion. The exact definition of the time of core formation,  $t_c$ , in model 1 and mass of core,  $\beta_c$ , in model 2, is ultimately dependent on and determined by the rate of Pb exchange between metallic and silicate phases at the pressures and temperatures prevalent for each point in the protomantle. If Pb exchange with the surrounding silicate phase is negligibly small when segregated metallic bodies reach, for example, metre scale, then this is the time measured by  $t_c$  (model 1); subsequent growth to larger units and shifts in their relative position to the centre of the Earth will not be measured. Therefore  $t_c$  will be a maximum time for the actual core formation. Similarly, in model 2 all (in this example) metre-sized or



**Fig. 3** Model 2: *a*, growth of relative mass of core,  $\beta_t$ , with time for three values of the core growth constant  $\epsilon$  obtained for core-mantle partition coefficients of 5, 3, and 2. *b*, Change of the mantle  $\mu$  value with time for  $D_{Pb}^{c/m} = 5, 3$ , and 2. The bulk Earth  $\mu$  value if primarily dependent on the core-mantle Pb partition coefficient.

larger metallic bodies would be included in the quantity  $\beta_t$ , regardless of whether they were uniformly distributed within the protomantle, or formed a layer, or were part of the actual core.

The shortcomings of both these simple models are severe: for example, energy released during core formation and cooling only on the surface of the Earth will cause non-uniform and time-variant temperature distribution, only the latter is taken into account in model 1, though not in model 2. Non-uniform temperature distribution will mean that partial segregation of the metallic phase may occur in some parts of the Earth earlier than in others. Then the calculated time of core formation in model 1 will become an average value. In model 2 this will cause the rate of core growth  $\epsilon$  to be variable in time. Therefore both models are oversimplifications which may only serve to demonstrate that there was probably a minimum time interval of some hundred Myr between accretion and completion of core-formation, but which cannot provide any detailed model of the process; such a model would have to take account of non-uniform temperature distribution.

Model 1 would entail the core being in equilibrium with the mantle for all non-radiogenic elements; from model 2 it would follow that the total core is now in disequilibrium with respect to all elements. This disequilibrium may easily be calculated from equations (2) and (11) and acts in such a way that all elements which partition into the core are depleted in the mantle below their equilibrium abundance and vice versa. It seems, however, that siderophile elements are on the contrary less depleted in the mantle

than demanded by equilibrium considerations<sup>18,19</sup>. This apparently leads to a paradox. Enrichment in the mantle of elements which are expected to partition into the core seems to demonstrate disequilibrium between core and mantle, on the other hand radiogenic Pb suggests that equilibrium between core and mantle had once been reached. Assuming that this disequilibrium is real and not just an indication of our lack of knowledge of distribution coefficients at high pressures and temperatures, a solution might be that temperatures within a considerable part of the Earth have never been sufficient for a metallic phase to separate. After core formation, however, this primordial material must have been thoroughly homogenised with depleted mantle to account for the radiogenic Pb excess. It is not possible to attribute this low temperature, primordial material to a chilled outer surface: a chilled protocrust would be expected to have a low  $\mu$  value and would thus be depleted in  $^{207}\text{Pb}$  for any given  $^{206}\text{Pb}$  value relative to the mantle. On the contrary, the continental crust is enriched in  $^{207}\text{Pb}$ . But Ringwood's model<sup>18</sup> of accretion and core formation would (with the exception of the time of core formation) fit these constraints. The interior of the Earth accretes cool, a metal phase separates only from the hot outer regions and collects into bodies which sink to the centre without attaining equilibrium with the material which they pass through. Subsequently, depleted and undepleted mantle regions were homogenised by convection. The  $^{207}\text{Pb}$  excess in the continental crust may indicate that the major part of the crust was formed from depleted mantle before homogenisation was achieved.

A time interval of some hundred Myr does not invalidate the precipitation hypothesis<sup>20</sup> for the origin of the moon. Although Ringwood states<sup>20</sup> that the moon forms after separation of the Earth's core, there is no reason why this has to be so, as long as the surface temperature on the Earth during the last stages of accretion is sufficiently high to evaporate silicates but not high enough to volatilise iron<sup>18</sup>.

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1. Cumming, G. L. & Richards, J. R. *Earth planet. Sci. Lett.* **28**, 155-171 (1975).
2. Richards, J. R. *Econ. Geol.* **66**, 425-434 (1971).
3. Doe, B. R. & Stacey, J. S. *Econ. Geol.* **69**, 757-776 (1974).
4. Wedepohl, K. H. in *Handbook of Geochemistry* (Springer, Berlin, 1974).
5. Tera, F., Papanastassiou, D. A. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **22**, 1-21 (1974).
6. Wasserburg, G. J., MacDonald, G. J. F., Hoyle, F. & Fowler, W. A. *Science* **143**, 465 (1964).
7. Taylor, S. R. *Geochim. cosmochim. Acta* **28**, 1273-1285 (1964).
8. Tatsumoto, M. *Science* **153**, 1094-1101 (1966).
9. Church, S. E. & Tatsumoto, M. *Contrib. Mineral. Petrol.* **53**, 253-279 (1975).
10. Jaffey, A. H., Flynn, K. F., Glendenin, L. E., Bentley, W. C. & Essling, A. M. *Phys. Rev. C* **4**, 1889-1906 (1971).
11. Richard, P., Shimizu, N. & Allegre, C. *Earth planet. Sci. Lett.* **31**, 269-278 (1976).
12. DePaolo, D. J. & Wasserburg, G. J. *Geophys. Res. Lett.* **3**, 743-746 (1976).
13. O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. *Earth planet. Sci. Lett.* **34**, 13-22 (1977).
14. Oversby, V. M. & Ringwood, A. E. *Nature* **234**, 463-465 (1971).
15. Brett, R. *Rev. Geophys. Space Phys.* **14**, 375-383 (1976).
16. Gale, N. H. & Mussett, A. E. *Rev. Geophys. Space Phys.* **11**, 37-86 (1973).
17. Tatsumoto, M., Knight, R. J. & Allegre, C. L. *Science* **180**, 1279-1283 (1973).
18. Ringwood, A. E. *Geochim. cosmochim. Acta* **30**, 41-104 (1966).
19. Kimura, K., Lewis, R. S. & Anders, E. *Geochim. cosmochim. Acta* **38**, 683-702 (1974).
20. Ringwood, A. E. *Earth planet. Sci. Lett.* **8**, 131-140 (1970).
21. Sun, S. S. & Jahn, B.-M. *Nature* **255**, 527-530 (1975).
22. Sun, S. S., Tatsumoto, M. & Schilling, J.-G. *Science* **190**, 143-147 (1975).
23. Sun, S. S. & Hanson, G. N. *Contrib. Mineral. Petrol.* **52**, 77-106 (1975).
24. Cumming, G. L. *Earth planet. Sci. Lett.* **31**, 179-183 (1976).
25. Oversby, V. M. *Geochim. cosmochim. Acta* **36**, 1167-1179 (1972).
26. Unruh, D. M. & Tatsumoto, M. in *Init. Rep. DSDP 34*, (Washington, 1976).
27. Reynolds, P. H. & Dasch, E. J. *J. geophys. Res.* **76**, 5124-5129 (1971).

# Neurotransmitter synthesis and uptake by isolated sympathetic neurones in microcultures

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*Assays of isolated single sympathetic neurones show that their transmitter functions can be either adrenergic or cholinergic depending on growth conditions. The data suggest that the number of transmitters made by most mature individual neurones is restricted.*

DEVELOPMENT of transmitter functions by primary sympathetic neurones cultured *in vitro* is remarkably plastic<sup>1-3</sup>. When grown alone in certain conditions, rat sympathetic neurones become primarily adrenergic. They develop the functions necessary for synthesis, uptake, storage, and release of catecholamines, but show relatively low levels of cholinergic function<sup>4-6</sup>. These





neurones can form morphological synapses on each other which contain high concentrations of small granular vesicles<sup>7-9</sup> but show no physiological activity, probably because the neurones are not very sensitive to catecholamines<sup>10</sup>. Cultures of these same neurones, grown with large numbers of non-neuronal cells, however, develop many cholinergic properties. They synthesise acetylcholine (ACh) and form functional cholinergic synapses with each other, heart, and skeletal muscle<sup>1-3, 11-15</sup>.

Medium 'conditioned' by incubation for 2 d with appropriate non-neuronal rat cells can also induce ACh synthesis and cholinergic synapse formation<sup>5, 9, 16, 17</sup>. The extent of ACh synthesis and cholinergic synapse formation increases as the neurones are exposed to higher concentrations of conditioned medium<sup>9, 16, 17</sup>. In contrast, development of the functions necessary for catecholamine synthesis and accumulation is repressed in a dose-dependent fashion by increasing concentrations of conditioned medium<sup>16</sup>. Among the neurones that establish themselves in culture there is little cell death and different doses of conditioned medium do not significantly change the number of neurones that survive<sup>16, 18</sup>. Therefore, it seems likely that a single population of immature sympathetic neurones is able to differentiate in more than one direction. By examining single cells, we hoped to determine how the development of transmitter function is regulated in individual neurones. In particular, is the population shifted from primarily single adrenergic neurones to primarily single cholinergic neurones by growth in high concentrations of conditioned medium? In the intermediate conditions do most single neurones simultaneously express both cholinergic and adrenergic functions? Our biochemical studies on these questions have been reported in preliminary form<sup>19</sup>. Motivated by similar objectives, Furshpan *et al.*<sup>12</sup> and Landis<sup>8</sup> have recently made physiological and morphological observations on individual neurones grown in conditions similar to ours.

### Synthesis of transmitter

When primary sympathetic neurones were plated at low density into micro-wells grown in conditioned medium lacking the cholinergic 'factor', and examined after 4 weeks, many wells were found to lack neurones. Others contained one visible neurone (Fig. 1), while still others contained 2-20 neurones. If only single neurones were plated initially, a Poisson distribution of neurones per well could be anticipated. In fact, small clusters of neurones were also plated so a typical plate contained 35 empty wells, 2-5 wells with single neurones, and 20 wells with more than one neurone.

**Fig. 1** An isolated sympathetic neurone. This montage shows an isolated neurone, grown on a dried collagen drop, with its neuritic ramifications. To assay transmitter synthesis the neurones were grown in Falcon 3034 Terasaki culture plates. One of these plates contains 60 wells with a capacity of 10  $\mu$ l each. Approximately 50  $\mu$ g of rat tail collagen in 10  $\mu$ l 0.1% acetic acid<sup>20</sup> was evaporated in each well. The plates were then sterilised under an ultraviolet lamp. Sympathetic neurones were dissociated from newborn rats (Charles River CD)<sup>4, 21</sup>. In some experiments, these were plated at appropriate dilutions directly into the micro-wells and allowed to settle for 2 h before conditioned medium (CM) or control L-15 CO<sub>2</sub> medium with methocel, rat serum, and nerve growth factor flooded the wells<sup>4, 16</sup>. In other experiments, cardiac cells were dissociated from the hearts of newborn rats with collagenase (EC 4.24.3) (Worthington type I, 1 mg ml<sup>-1</sup> in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Hanks solution for 20 min at 37 °C) and approximately 500 cells were seeded in each well. After 2 h, the wells were flooded with complete L-15 CO<sub>2</sub> medium containing rat serum, but lacking methocel and nerve growth factor. After 1-4 d, the heart cells had formed monolayers in the wells. At this point, the wells were seeded with neurones at an appropriate dilution and incubated with complete L-15 CO<sub>2</sub> medium<sup>4, 16</sup> containing methocel, rat serum and nerve growth factor. In all our cultures, proliferation of heart and other non-neuronal cells was prevented by  $\gamma$ -irradiation (5,000 rad over 25 s) 2 d after the neurones were seeded. Neurones adhering to the sides of these wells were scraped off 2 d after plating with a tapered Teflon rod. Cells were grown for 28-35 d in the same L-15 CO<sub>2</sub> medium, which was changed on day 2 and every 4 d thereafter. The wells were then scanned with the phase contrast optics and only those in which the neurones could be counted easily were used for studies of transmitter synthesis and uptake. In preparation for labelling, the plates were washed twice with complete L-15 CO<sub>2</sub> medium lacking methocel and three times with complete L-15 CO<sub>2</sub> medium lacking methocel, tyrosine, and choline. The neurones were starved for 30 min in this medium and the plates washed three times more with complete L-15 medium lacking methocel, tyrosine and choline. At this point 10  $\mu$ l of this same medium, containing 0.25  $\mu$ g freshly added ascorbate, 700 pmol <sup>3</sup>H-L-tyrosine (10-20 Ci mol<sup>-1</sup>), and 700 pmol <sup>3</sup>H-choline (10.1 Ci mmol<sup>-1</sup>), were added to each well. At 4 and 8 h later, an additional 1  $\mu$ l of the same medium, containing 0.25  $\mu$ g fresh ascorbate, was added to each well. After 12 h incubation at 37 °C, the plates were washed three times more with the same medium, without isotope. The wells were drained, and each well was filled with 10  $\mu$ l of pH 1.9 buffer containing unlabelled standards<sup>4, 22</sup>. The plates were stored for 1-48 h at -20 °C and wells were thawed and frozen twice before the contents were applied to paper, and the transmitters were separated by high voltage electrophoresis (6,000 V, 90 min)<sup>22</sup>. The sections of paper containing dopamine, NA and ACh were identified<sup>22</sup>, cut out, and counted<sup>4</sup>. The counting efficiency (~25%) was determined by drying on paper known quantities of the tritiated precursors. CM containing the 'cholinergic factor' was obtained by incubating 20 ml of complete L-15 CO<sub>2</sub> medium for 2 d with a 75-cm<sup>2</sup> monolayer of the rat glioma cell line C6 (ATCC CCL107<sup>23</sup>) in a Falcon 7004 tissue cultured flask. CM lacking the 'factor' was obtained by incubating the same amount of medium with a monolayer of embryonic chicken heart cells in the same type of flask<sup>16</sup>. The chicken heart cells were obtained from 10-d chicken embryos by collagenase dissociation, using the procedure used for rat hearts. Single neurones are apparently more fastidious than mass neuronal cultures; so that standard culture conditions used in these studies were not always successful. It was particularly difficult to obtain firm adhesion of single neurones to collagen sheets. All manipulations had to be performed very gently, and it was useful to verify that the neurone remained in the well at the end of the many medium changes that followed the incubation with labelled precursors. Since 1 fmol of labelled transmitter provided only 10-20 c.p.m., it was important to clean the electrophoresis tanks before each run and to use standards, blotting paper, scissors, and scintillation fluid that had not been contaminated by other experiments. With these precautions, the background was reduced to approximately 30 c.p.m. in favourable experiments. Unknown causes gave higher backgrounds in some experiments and these results were discarded. All vials were counted twice for 10 min and the results were averaged. The pieces of paper containing transmitters were compared to equal sized paper pieces, cut just in front and behind the transmitter spots.

Most of these wells were incubated with <sup>3</sup>H-tyrosine and <sup>3</sup>H-choline for 12 h and synthesis of <sup>3</sup>H-ACh and <sup>3</sup>H-noradrenaline (NA) was measured after the incubation. The results of a typical experiment are presented in Fig. 2. Significant amounts of <sup>3</sup>H-ACh were not detected in any well. Synthesis of <sup>3</sup>H-NA was not detected in any wells lacking visible neurones. In the 10 wells containing a single neurone, however, 10-50 fmol of NA synthesis was seen. Wells containing more neurones made, on the average, more <sup>3</sup>H-NA. (Only the results from the wells containing 0, 1 and 4 and 5 neurones are presented in Fig. 2 because the other classes provided no additional insight.) These results show that single neurones

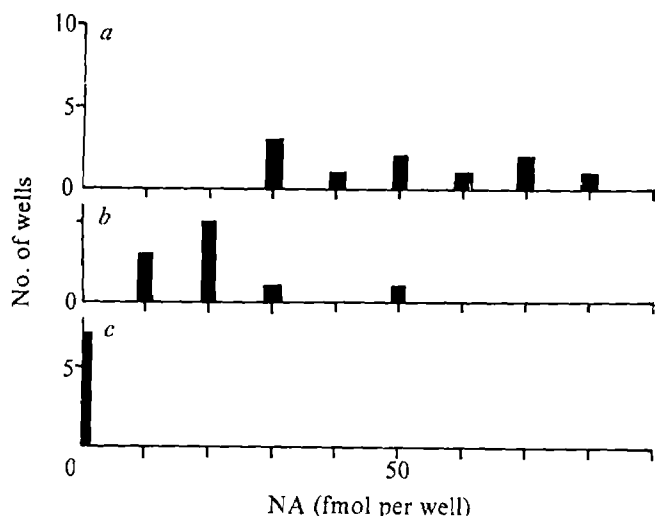


Fig. 2  $^3\text{H}$ -ACh and  $^3\text{H}$ -NA synthesis by neurones grown in control medium. The neurones were grown for 29 d in complete L-15  $\text{CO}_2$  medium supplemented with 20% of the same medium first conditioned on a monolayer of chicken embryo heart cells. The neurones in each well were counted and incubated with  $^3\text{H}$ -choline and  $^3\text{H}$ -tyrosine. The results are plotted in separate graphs for those wells containing: a, 0; b, 1, or c, 4 or 5 neurones. No synthesis of detectable  $^3\text{H}$ -ACh was seen in any well. The amount of  $^3\text{H}$ -NA synthesised rounded to the nearest multiple of 10 fmol, is plotted on the abscissa. The number of wells making a particular amount of  $^3\text{H}$ -NA is plotted on the ordinate.

make detectable quantities of  $^3\text{H}$ -NA, but not  $^3\text{H}$ -ACh, when grown in these conditions. Every neurone synthesised and accumulated detectable transmitter, but the amount made by a single cell may differ by at least a factor of 5.

Why do single neurones make such differing quantities of  $^3\text{H}$ -NA? To see whether differences in transmitter synthesis simply reflected differences in metabolic activity of neurones, we measured the acid precipitable radioactivity in  $^3\text{H}$ -tyrosine and  $^3\text{H}$ -choline derivatives. The results are shown in Fig. 3. There is a correlation, but not a strong one between the amounts of radioactivity in the acid precipitable and NA fractions. Some neurones were metabolically active, but produced relatively little transmitter. By this criterion, the neurones seem to have differentiated to different extents after culture for 4 weeks *in vitro*. Immature neurones have been seen in superior cervical ganglia as late as 3 weeks after birth<sup>24</sup>. Our results could reflect this documented *in vivo* heterogeneity, but alternatively, may reflect limitations in our methods for nerve culture.

The primary sympathetic neurones can also be grown in conditions which promote cholinergic development. Figure 4 shows the results of one representative experiment in which the neurones were plated onto monolayers of rat embryo heart cells and labelled 32 d later with  $^3\text{H}$ -tyrosine and  $^3\text{H}$ -choline. Wells containing heart cells but not neurones made neither  $^3\text{H}$ -NA nor  $^3\text{H}$ -ACh. In the wells containing neurones, the average synthesis of  $^3\text{H}$ -ACh was seven times that of  $^3\text{H}$ -NA, but single neurones did not synthesise both transmitters in this ratio. In 26 of the 30 wells that seemed to contain a single neurone we detected synthesis of

8–53 fmol of  $^3\text{H}$ -ACh without detectable  $^3\text{H}$ -NA synthesis. In three wells there was synthesis of 9–17 fmol of  $^3\text{H}$ -NA but no detectable  $^3\text{H}$ -ACh. In only one single neurone well was there synthesis of both  $^3\text{H}$ -ACh and  $^3\text{H}$ -NA, 6 fmol and 3 fmol respectively. The results suggest that there are at least two and possibly three classes of neurones, differing in their patterns of transmitter synthesis. Support for this interpretation is provided by the data obtained from wells containing more than one neurone. Synthesis of  $^3\text{H}$ -ACh was seen in all five wells containing two neurones, but  $^3\text{H}$ -NA was seen in only one, suggesting that four wells contained two cholinergic neurones while one well may have contained one adrenergic and one cholinergic neurone. In the wells containing more neurones, highly disparate ratios of  $^3\text{H}$ -ACh to  $^3\text{H}$ -NA synthesis were seen. These fluctuations also suggest that more than one class of neurones developed in these wells.

Several experiments have been done on single cells grown in conditions where the average ratio of  $^3\text{H}$ -NA synthesis is closer to one. In these experiments, the neurones developed in the presence of L-15  $\text{CO}_2$  medium conditioned by previous growth with the rat glioma cell line C6. Neuronal mass culture grown in this medium make significant amounts of  $^3\text{H}$ -ACh<sup>16</sup>. The results with the single neurones are summarised in Table 1. In 50% conditioned medium approximately 45% of the neurones made detectable levels of  $^3\text{H}$ -ACh, but not  $^3\text{H}$ -NA; roughly the same percentage made  $^3\text{H}$ -NA, but not  $^3\text{H}$ -ACh. In about 5% of the wells synthesis of both transmitters was seen. In 20% conditioned medium, 90% of the neurones were adrenergic; the remainder were cholinergic. The

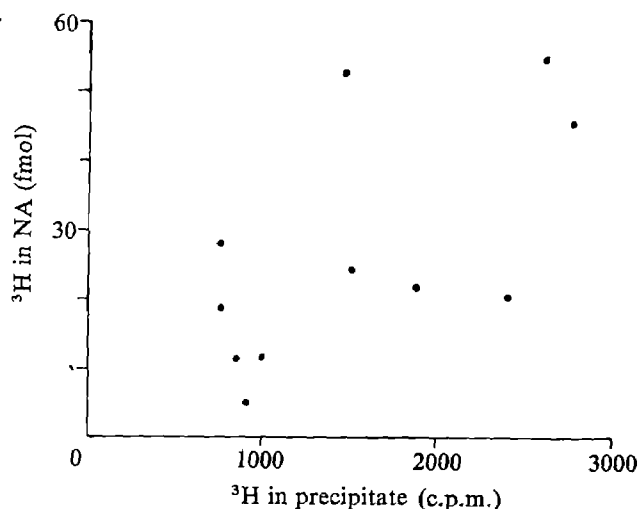
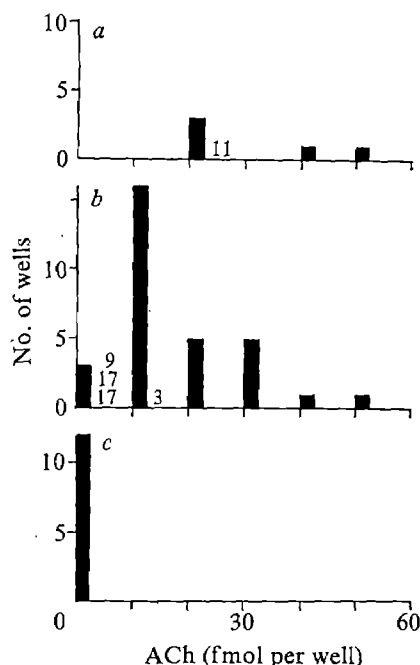


Fig. 3 Incorporation of radioactivity into the acid-precipitable and NA fractions of single neurones. The cells from Fig. 1 were used for this experiment. After high voltage electrophoresis, strips of paper including the regions within 1 cm of the origin were placed on a Millipore filter with the sides to which radioactivity had been applied uppermost. These strips were washed with cold 10% trichloroacetic acid, and counted in a scintillation counter to determine the amount of acid precipitable radioactivity. The background from five wells containing no neurones averaged 42 c.p.m. and was subtracted. c.p.m. acid precipitable radioactivity is plotted on the ordinate; fmol  $^3\text{H}$ -NA in the precipitate on the abscissa.

Table 1 Transmitter synthesis by single neurones grown in various conditions

Growth conditions	ACh neurones (a)	NA neurones (b)	ACh and NA neurones (c)	Negative neurones (d)	ACh:NA ratio
Control	0	18	0	0	<0.02
20% CM	2	21	0	0	0.15
50% CM	15	15	2	2	2.4
Heart cells	26	3	1	0	7.0

Pooled results are given of all experiments on neurones grown in control medium, in 20% and 50% conditioned medium (CM), and on rat heart monolayers. Procedures for growing neurones in control medium and on rat heart monolayers were as described in Figs 1 and 3. Neurones grown with 20% or 50% conditioned medium (conditioned with C6 cells) were labelled 25–28 d after plating. The values are the numbers of single neurones making only ACh (a), the number making only NA (b), the number making ACh and NA (c), the number making neither transmitter (d), and the average ratio of ACh to NA synthesis in the pooled results, including wells with more than one neurone.



**Fig. 4**  $^3\text{H}$ -ACh and  $^3\text{H}$ -NA synthesis by neurones grown on heart cells. Culture age was 32 d. The neurones in each well were first counted, then the wells were washed and incubated with  $^3\text{H}$ -choline and  $^3\text{H}$ -NA (see Fig. 1 legend). The results are summarised in separate graphs for wells containing 0 (c), 1 (b), or 2 (a) neurones. The fmol  $^3\text{H}$ -ACh synthesised by a particular well is plotted on the abscissa. The number of wells in which there was a particular amount of  $^3\text{H}$ -ACh synthesis is plotted on the ordinate. Values for  $^3\text{H}$ -ACh synthesis are rounded to the nearest multiple of 10 fmol. (There were no examples, though, in which 2–5 fmol  $^3\text{H}$ -ACh was synthesised, and only one example in which a cholinergic neurone made less than 8 fmol  $^3\text{H}$ -ACh. That neurone seemed to make 6 fmol  $^3\text{H}$ -ACh and 3 fmol  $^3\text{H}$ -NA). When there was detectable  $^3\text{H}$ -NA synthesis in a well, the amount has been written directly above or to the right of the appropriate bar. Averaged,  $^3\text{H}$ -ACh synthesis by all these neurones was seven times as high as  $^3\text{H}$ -NA synthesis.

pooled results of all our experiments are summarised in Table 1. It seems that most cells become primarily cholinergic or adrenergic, but that the percentage committed to either path depends on the growth conditions.

### Uptake of noradrenaline

Transmitter synthesis is only one parameter of neuronal function. Many neurones also have high affinity uptake systems for the transmitters that they synthesise, store, and release (compare ref. 25). We have found that sympathetic neurones cultured in conditions which promote adrenergic development possess such an uptake system for NA, with a  $K_m$  of  $\sim 1 \mu\text{M}$ , which is blocked by cocaine. Storage of this NA is abolished by reserpine<sup>5</sup>. Mass cultures of sympathetic neurones grown on monolayers of rat heart retained the ability to concentrate and store  $^3\text{H}$ -NA, but at a reduced rate (data not shown). One possibility was that no  $^3\text{H}$ -NA uptake occurred in neurones making ACh.

To see whether cholinergic neurones could also concentrate and store NA, single neurones were grown on rat heart monolayers for 30 d and were then labelled with  $^3\text{H}$ -choline, and  $^3\text{H}$ -NA (50 nM). The results are presented in Fig. 5. Wells containing no neurones neither made  $^3\text{H}$ -ACh nor concentrated  $^3\text{H}$ -NA. Significant  $^3\text{H}$ -NA uptake was seen in all 57 wells containing a single neurone;  $^3\text{H}$ -ACh synthesis was seen in 53 of these same wells. Clearly, most neurones have an uptake system for NA. The few that did not do both may have been cells, like those in Fig. 4, that synthesised only  $^3\text{H}$ -NA and not  $^3\text{H}$ -ACh.

### Conclusion

Examination of transmitter synthesis and uptake functions in developing sympathetic neurones has revealed the existence of two or three different classes of cells. The first class, virtually the only

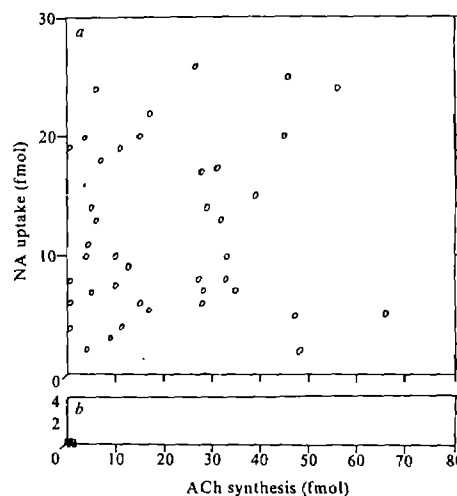
class present when the neurones are grown alone, is adrenergic. The individual neurones synthesise considerable NA, but no detectable ACh. In mass culture, they have a high affinity uptake system for NA<sup>5,6</sup>. They almost certainly correspond to the neurones described by Furshpan *et al.* and Landis<sup>1,2,8</sup> that, when stimulated, evoked only adrenergic responses in heart cells and have varicosities filled with small granular vesicles after  $\text{KMnO}_4$  fixation to localise endogenous NA.

The second class synthesises considerable amounts of ACh, but no detectable NA; however, these neurones retain the ability to concentrate and store exogenous NA (Fig. 5). These probably correspond to the neurones described by Furshpan *et al.*<sup>12</sup> and Landis<sup>8</sup> which contained no endogenous small granular vesicles, formed cholinergic synapses on themselves and evoked cholinergic but not adrenergic responses in heart muscle cells.

Furshpan *et al.*<sup>12</sup> and Landis<sup>8</sup> have also presented convincing evidence for the presence in young cultures of a third class of neurones that releases both cholinergic and adrenergic transmitters on heart muscle cells. Their experiments did not show that single cells synthesised both transmitters; conceivably they could have concentrated NA made by other neurones on the dish. They also did not show whether the neurones were transiently or permanently bifunctional. It is possible that all young neurones responding to the cholinergic inducer substance(s) might pass through a bifunctional phase. Our experiments with more mature neurones do not contribute to the study of these very interesting cells. Although we have found occasional neurones that seemed to synthesise both NA and ACh (Fig. 4, Table 1), they were rare enough to be possible artefacts. Either a hidden neurone or terminals from one whose cell body had been lost might explain our results. Neuronal numbers can be counted with high, but not absolute, reliability with phase contrast optics. This limitation in our methods makes it difficult to study rare classes of neurones. In the future, of course, neurones shown electrophysiologically to be releasing both ACh and NA can also be assayed for ACh and NA synthesis from labelled precursors.

The two major classes, those neurones exhibiting cholinergic and adrenergic biosynthetic activities, are present in different proportions in different growth conditions. In our experiments, the fraction of neurones committed to ACh synthesis increased as the ratio of ACh to NA synthesis in the whole population increased (Table 1). In addition to the shift in the population of neurones caused by conditioned medium or heart cells, it is also possible that these 'cholinergic' factor(s) when present in higher concentrations,

**Fig. 5**  $^3\text{H}$ -NA uptake and  $^3\text{H}$ -ACh synthesis by the same neurones. Neurones were grown for 30 d in complete L-15  $\text{CO}_2$  on monolayers of embryonic rat heart cells. The neurones in each well were counted. Then they were incubated with  $^3\text{H}$ -choline and unlabelled tyrosine for 10 h. Each well then received  $2 \mu\text{l}$  of the same medium containing 50 fmol  $^3\text{H}$ -NA (22 Ci  $\text{mmol}^{-1}$ ; NEN). The final  $^3\text{H}$ -NA concentration was 50 nM. Wells were washed and cells collected 2 h later. The results are for wells containing 0 (b) or 1 (a) neurone. The fmol  $^3\text{H}$ -ACh synthesised is on the abscissa; the fmol  $^3\text{H}$ -NA uptake is on the ordinate. Each circle represents a single well.



might cause neurones committed to the cholinergic decision to produce higher levels of ACh. The heterogeneity in the individual neuronal responses in our experiments precluded a firm answer on this point.

Virtually every single neurone that survived made at least one transmitter in our experiments. This result suggests that there are no large populations of 'silent' neurones making neither ACh nor NA in mass cultures grown in adrenergic or cholinergic conditions. Since the same number of neurones survive in cultures grown in several different concentrations of cholinergic 'factor'<sup>16</sup>, selective death of adrenergic cells in cholinergic conditions and the reverse are unlikely to explain the alterations in transmitter synthesis. Instead, individual neurones probably retain developmental plasticity at the time they are plated on the dish.

Most primary sympathetic neurones did not make detectable amounts of ACh and/or NA (Figs 2,4; Table 1), suggesting that regulatory mechanisms limit transmitter synthesis by single sympathetic neurones. Such mechanisms may force individual cells to respond in a 'flip-flop' fashion to the cholinergic 'factor'. The young dual functional neurones described by Furshpan *et al.*<sup>12</sup> may have been in a transitional state when they were examined. This may also have been true for the few neurones that we observed which made two transmitters. The heterogeneity in response of the individual neurones to a given level of the cholinergic 'factor' may reflect the heterogeneity in developmental time course that has been documented *in vivo*<sup>24</sup>, or it could reflect microheterogeneity in the tissue culture environment. Either could explain the broad distribution in rate of transmitter synthesis by single neurones.

In some cells, however, regulation of transmitter synthesis is more complex than predicted by the 'flip-flop' model. In several systems there is evidence for the simultaneous presence of more than one transmitter in a single neurone<sup>26</sup>. Neuroblastoma clones have been isolated which contain high levels of tyrosine hydroxylase and choline acetyltransferase<sup>27</sup>. Similarly, the rat pheochromocytoma PC12 synthesises both NA and ACh<sup>28-30</sup>. Some, but not all sub-clones of PC12, retain high levels of both tyrosine hydroxylase and choline acetyltransferase. While single cell analysis has not been done on this clonal line, the available results make it unlikely that cell lines are forced to respond in a 'flip-flop' fashion. Possibly, the clonal lines are frozen in an embryonic state of development. Alternatively, choices between transmitters are not obligatory, but are highly probable in the conditions in which the cultured sympathetic neurones develop.

The uptake of NA by neurones that synthesise ACh is consistent with the observation that NA can be concentrated into small granular vesicles of identified single neurones that secrete ACh<sup>8</sup>. Experiments on mass cultures had indicated that NA uptake was reduced 10-20-fold in cholinergic growth conditions (data not shown), so we had expected NA uptake to be restricted to adrenergic cells. Possibly the proteins required for the uptake and storage of NA are regulated independently of the NA biosynthetic

enzymes. Indeed, neuroblastoma clones that have one, but not another of the biosynthetic enzymes for NA have been described<sup>31</sup>, suggesting that every adrenergic function can be independently regulated. Alternatively, all the sympathetic neurones may develop some adrenergic functions before they respond to the cholinergic 'factor'. In support of this interpretation, sympathetic cells in newborn rats are known to be weakly fluorescent<sup>24</sup> and to initially form *in vitro* endings with high concentrations of small granular vesicles which are lost with cholinergic development<sup>3</sup>. At least one sympathetic neurone-like rat pheochromocytoma clone also synthesises significant amounts of NA in the 'undifferentiated' state<sup>28</sup>. Possibly, then, the proteins required for synthesis, uptake, storage, and release of NA are made in most young sympathetic neurones. Their synthesis is reduced or eliminated by contact with the cholinergic 'factor', but the activities which are more stable persist in lower amounts in the developing cholinergic cell.

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1. O'Lague, P., Obata, K., Claude, P., Furshpan, E. & Potter, D. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3602-3606 (1974).
2. Patterson, P. & Chun, L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3607-3610 (1974).
3. Johnson, M. *et al. Nature* **262**, 308-310 (1976).
4. Mains, R. & Patterson, P. *J. Cell Biol.* **59**, 329-345 (1973).
5. Patterson, P., Reichardt, L. & Chun, L. *Cold Spring Harb. Symp. quant. Biol.* **40**, 389-397 (1975).
6. Burton, H. & Bunge, R. *Brain Res.* **97**, 157-162 (1969).
7. Rees, R. & Bunge, R. *J. comp. Neurol.* **157**, 1-12 (1974).
8. Landis, S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4220-4224 (1976).
9. Landis, S., MacLeish, P., Furshpan, E., Potter, D. & Patterson, P. *6th Ann. Soc. Neurosci. Abstr.* **280** (1976).
10. Obata, K. *Brain Res.* **73**, 71-88 (1974).
11. Nurse, C. & O'Lague, P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1955-1959 (1975).
12. Furshpan, E., MacLeish, P., O'Lague, P. & Potter, D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4225-4229 (1976).
13. Ko, C.-P., Burton, H., Johnson, M. & Bunge, R. P. *Brain Res.* **117**, 461-485 (1976).
14. Crain, S. & Peterson, E. *Ann. N. Y. Acad. Sci.* **228**, 6-34 (1974).
15. Purves, R. *et al. Pflügers Arch.* **350**, 1-7 (1974).
16. Patterson, P. & Chun, L. *Dev. Biol.* **56**, 263-280 (1977).
17. MacLeish, P. thesis, Harvard Univ. (1976).
18. Chun, L. & Patterson, P. *J. Cell Biol.* (in the press).
19. Reichardt, L., Patterson, P. & Chun, L. *6th Ann. Soc. Neurosci. Abstr.* **274** (1976).
20. Bornstein, M. B. *Lab. Invest.* **7**, 134-140 (1958).
21. Bray, D. *Proc. natn. Acad. Sci. U.S.A.* **65**, 905-910 (1970).
22. Hildebrand, J., Barker, D., Herbert, E. & Kravitz, E. *J. Neurobiol.* **2**, 231-246 (1971).
23. Benda, P., Lightbody, J., Sato, G., Levine, L. & Sweet, W. *Science* **161**, 370-371 (1968).
24. Eränkö, L. *Brain Res.* **46**, 159-175 (1972).
25. Iversen, L. *The Uptake and Storage of Catecholamines* (Cambridge University Press, Cambridge, 1967).
26. Burnstock, G. *Neuroscience* **1**, 239-248 (1976).
27. Prasad, K. *et al. Nature new Biol.* **241**, 117-119 (1973).
28. Greene, L. & Tishler, A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2424-2428 (1976).
29. Shubert, D., Heinemann, S. & Kidokoro, Y. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2579-2583 (1977).
30. Greene, L. A. & Rein, G. *Nature* **268**, 349-351 (1977).
31. Hamprecht, B., Traber, J. & Lamprecht, F. *FEBS Lett.* **42**, 221-226 (1974).

## Detection of acceptor sites on human lymphocytes for antigen-specific T cell factors

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*Mouse antigen-specific T cell factors are absorbed by human peripheral blood lymphocytes at acceptor sites. The acceptors are products of HLA-linked genes, which may be human immune response genes.*

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THE immune response or Ir genes control the levels of immune responses to specific antigens. In many species, an important group of such genes has been shown to lie in the major histocompatibility complex (MHC) (ref. 1). Considerable interest attaches to the Ir genes of man, in particular because of the associations between antigens of the HLA system (the human MHC) and certain diseases, many of an autoimmune character, such as ankylosing spondylitis, Addison's disease, juvenile diabetes, coeliac disease and multiple sclerosis<sup>2,3</sup>. Ir gene polymorphism is



thought to play an important part in these disease associations. Some progress has been made in demonstrating human Ir genes, particularly in certain allergies<sup>4-6</sup>, but a major obstacle in experimental work is the ethical and practical problem of immunising volunteers and their families with antigens of scientific interest. In order to avoid these difficulties, we have attempted to detect products of Ir genes on human lymphocytes *in vitro*, using methods which have been successful in the mouse. In particular we have looked for Ir gene products involved in immunological cell interactions. Two types of such products have been described recently, namely the antigen-specific T cell factors, which are soluble mediators of T cell-B cell<sup>7</sup> and T cell-T cell<sup>8</sup> interaction, and the cell-bound acceptors, the sites on the lymphocyte surface where reaction with the T cell factors takes place<sup>7</sup>. The factors include antigen-specific helper and suppressor products which are assayed by their abilities to replace the helper or suppressor effects of T cells *in vivo* or *in vitro*<sup>9-12</sup>, while the acceptors are defined by the capacity of lymphocytes to absorb the specific factors<sup>7</sup>. In the mouse, both the factors and the acceptors are coded at least partially by genes in the I region of the H-2 complex<sup>7,13,14</sup>, and it has been proposed that they are the structural products of the Ir genes which are found in the same region. Thus, the inherited absence of either the helper factor for a particular antigen or its acceptor, is associated with low response to that antigen in mice<sup>7</sup>. In this report we describe a remarkable feature of the mouse specific helper factors, namely their ability to be absorbed by human peripheral blood lymphocytes (PBL). On interaction with the mouse factor, human PBL are activated and proceed to antibody production *in vitro*<sup>15</sup>. This suggests that critical mechanisms for the interaction between helper and acceptor structures have been preserved in speciation. Furthermore, the acceptor sites of human PBL seem to be polymorphic: for antigens such as synthetic polypeptides under murine Ir gene control, lymphocytes of different human individuals can be shown to be either high or low absorbers of the relevant T cell factors. Family analyses indicate that the acceptor genes are linked to the HLA complex, and preliminary mapping of two loci has been achieved in HLA-recombinant families. These findings provide an approach to the detection and mapping of the Ir genes of man.

### T-cell factor interaction with human lymphocytes

Antigen-specific T cell helper factors were prepared against sheep erythrocytes (SRBC) and the synthetic polypeptides (T,G)-A-L (batch 1383) and (Phe,G)-A-L (batch 223). The method, detailed elsewhere<sup>7,9</sup>, involved *in vitro* incubation of specifically educated (primed) mouse (C57B1/6) thymocytes together with antigen for 6-8 h. The cell-free supernatants of the educated T-cell cultures were the source of T-cell factors. Factor activity was assayed, before and after absorption by human lymphocytes by transfer into irradiated (700 rad) syngeneic mouse recipients together with bone marrow cells and antigen<sup>7,9</sup>. Plaque forming cells (PFC) against the relevant antigen were measured 8-10 d later for SRBC or 12-14 d later for polypeptides, in the spleens of the animals, using appropriately coated erythrocytes<sup>9</sup> and compared with suitable controls. Donors of human lymphocytes were employees of Hoffman-La Roche, Basel, and members of their families. Samples of 20 ml of blood were taken in heparin and lymphocytes separated by Ficoll sedimentation<sup>16</sup>. The cells were washed three times in medium free of calcium and magnesium and maintained before use in phosphate buffered saline (PBS) containing 10% foetal calf serum. For absorption of T-cell factor, 10<sup>7</sup> PBL from each donor were sedimented and resuspended in 2-3 spleen equivalents of T-cell factor (approximately 0.5 ml of the supernatant of educated T-cell culture), without further addition of antigen. After incubation for 30 min in ice, the lymphocytes were removed by centrifugation and the absorbed supernatants were mixed with mouse bone marrow cells and antigen and transferred into irradiated recipients (five per group) as described above. All the absorption tests on human lymphocytes were carried out strictly blind, with the identity or HLA type of individuals concerned being revealed only after final results had been obtained. HLA-typing was by standard techniques, using the facilities of the Basel Institute for Immunology.

**Table 1** PFC responses before and after absorption of specific mouse T cell factors with lymphocytes of different human donors

PBL donor (initials)	Mouse factor specific for		SRBC
	(T,G)-A-L	(Phe,G)-A-L	
-(Controls)	2,100 ± 280	3,600 ± 312	4,400 ± 360
GE	10* ± 8	25* ± 15	71* ± 35
GM	1,700† ± 210	15* ± 11	40* ± 26
HB	15* ± 11	2,700† ± 188	55* ± 26
RF	2,400† ± 235	2,850† ± 296	112* ± 65

Results as geometric means of PFC per spleen ± s.d. (five mice per group).

\*High absorber.

†Low absorber.

Incubation of the antigen-specific mouse helper factors with human PBL results, in many cases, in the complete removal of factor activity from the supernatant. This phenomenon is consistently observed in randomly-selected human donors for the helper factor specific for SRBC. Table 1 shows a typical absorption result, in which 10<sup>7</sup> PBL of four donors all removed the SRBC-specific factor. No failure to absorb this factor has yet been observed. But, when the mouse factors specific for the synthetic polypeptides (T,G)-A-L and (Phe,G)-A-L were used, the PBL of some but not all donors were found to absorb. In the majority of cases, clear-cut distinction between high and low absorbers was possible. Table 1 illustrates this—PBL of donors GE and HB absorbed the (T,G)-A-L specific factor, whereas those of GM and RF failed to remove the factor. A similar result is seen for the (Phe,G)-A-L factor, where this time GE and GM are high absorbers, and HB and RF are low absorbers. It thus seems that acceptor sites for the (T,G)-A-L and (Phe,G)-A-L specific factors are expressed on the PBL of some but not all individuals, while the ability to absorb SRBC-specific factor has been found on PBL of all individuals so far tested. Moreover, as Table 1 shows, all combinations of high and low absorption seem to be possible in different individuals (with the exception of low to SRBC factor).

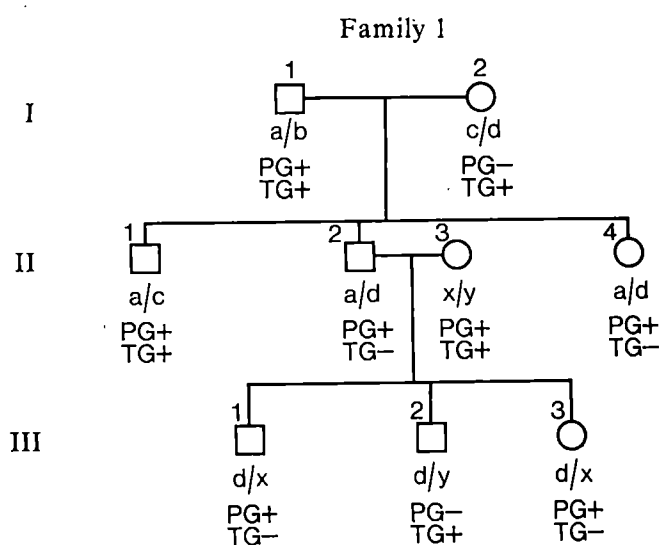
These findings have so far been extended to 41 random individuals, and the results are summarised in Table 2. Approximately 34% of the individuals tested are low absorbers of the (T,G)-A-L factor, 27% are low absorbers of the (Phe,G)-A-L factor, and 15% are low absorbers for both, although it should be noted that a smaller number of donors have so far been tested for (Phe,G)-A-L (26 tested) than for (T,G)-A-L (41 tested). These findings closely resemble those made in the mouse, where the B cells of some low responder strains fail to absorb the (T,G)-A-L or (Phe,G)-A-L specific factors and various combinations of the low absorption phenotypes for the different factors have been found. Note that in the mouse, lack of the acceptor site for an antigen-specific T cell factor can be correlated with low response of the animal to the antigen<sup>7</sup>. The absorption characteristic of each individual was a stable trait which could be detected very reproducibly, as indicated by perfect concordance in a re-test of 25 donors carried out blind, 6 months after first testing.

Family analysis strongly suggests that the abilities to absorb the T-cell factors for the two synthetic polypeptides are indeed inherited as simple Mendelian traits. A typical pedigree in three generations is shown in Fig. 1. This family illustrates the dominance of the high absorption characteristic. For example, the

**Table 2** Absorption of specific mouse T cell factors by human lymphocytes: summary of phenotypes observed

No. of phenotypes	(T,G)-A-L	Factor absorption (Phe,G)-A-L	SRBC
13	High	High	High
3	High	Low	High
11	High	NT	High
6	Low	High	High
4	Low	Low	High
4	Low	NT	High

Approximate gene frequencies: (T,G)-A-L, high 0.4, low 0.6; (Phe,G)-A-L, high 0.5, low 0.5 (see discussion). NT, not tested.



HLA haplotypes			Absorption loci	
A	B	C	<i>p</i>	<i>t</i>
(a) w30	12	w2	+	-
(b) w24	w35	w4	(?)	+
(c) w23	w17	-	-	+
(d) w32	w40	w3	-	-
(x) 1	8	-	+	-
(y) 29	12	-	-	+

**Fig. 1** Pedigree of abilities to absorb mouse T cell factors. PG+ and PG-, respectively the high and low absorption phenotypes for the (Phe,G)-A-L-specific mouse T cell factor. TG+ and TG-, respectively the high and low absorption phenotypes for the (T,G)-A-L-specific mouse T-cell factor. a, b, c, d, x and y, represent the HLA HLA haplotypes of the family members. *p* and *t*, the genetic loci controlling respectively the absorption of the (Phe,G)-A-L and (T,G)-A-L factors. With respect to the *p* and *t* loci, the signs + and - denote high and low absorption alleles respectively. The key shows the deduced linkage phase of *p* and *t* with each haplotype.

mating II.2 × II.3 between two PG+ individuals gives both PG+ and PG- offspring; this can be interpreted as an intercross between two heterozygotes of genotype  $p^+/p^-$ , in which the 'high absorber' gene  $p^+$  behaves as dominant over its  $p^-$  allele. A similar interpretation applies to the inheritance of the TG character in the offspring of the mating I.1 × I.2. Thus, family data support a model in which the absorption abilities are controlled by two loci (*t*, *p*) each with two alleles ( $t^+$ ,  $t^-$ ;  $p^+$ ,  $p^-$ ), high being dominant over low. Accepting this model and assuming that the population presented in Table 2 is at Hardy-Weinberg equilibrium, the gene frequencies are:  $t^+ \approx 0.4$ ,  $t^- \approx 0.6$ ;  $p^+ \approx 0.5$ ,  $p^- \approx 0.5$ .

### Acceptor relationship to HLA system

On the basis of the situation in the mouse it was obvious to look for a linkage with the HLA region, and indeed inspection of the family in Fig. 1 shows that the genes controlling the TG and PG phenotypes, assigned independently of any knowledge of the HLA typing, segregate with the HLA haplotypes. So far, no cross-over between HLA and the acceptor loci has been observed in over 20 potentially informative offspring of backcross type matings, (other than in families with known recombinations within the HLA complex as described below). Thus, close linkage between the acceptor loci *t* and *p*, and HLA is indicated, though a significant recombination fraction cannot be excluded. In this and other

families several other genetic markers were studied, either with no information (Kk, Kp, Js, Wr<sup>a</sup>, ADA, AK, Inv) or with evidence of independent segregation (ABO, MNSs, P, Rh, Fy, Lu, Kl, Le, Xg<sup>a</sup>, Hp, ACP<sub>1</sub>, PGM<sub>1</sub>, GPT, Gc, Pt and Gm).

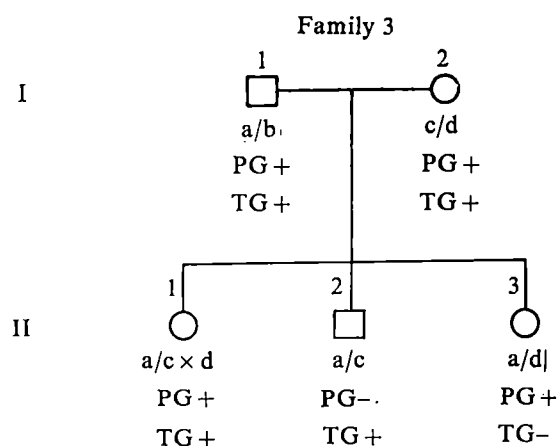
Confirmation that there are indeed two acceptor loci in the HLA complex comes from HLA-recombinant families, one of which is shown in Fig. 2. The recombinational event occurred between the HLA-A and HLA-C loci in the mother and the recombinant haplotype (denoted c × d) is carried by a daughter (II.1). This pedigree shows that crossing over must also have occurred between the acceptor loci *t* and *p*, and that the acceptor locus *t* must be on the HLA-A side of the cross-over event, while the acceptor locus *p* is on the HLA-C side. Figure 3 illustrates this in skeleton form. Further recombinant families are being studied to localise the acceptor genes further.

In some instances, for HLA antigens of the A, B, C series a trait under study is associated with a given marker at another locus of the HLA region, that is, the two traits are in linkage disequilibrium<sup>17</sup>. Analysis of over 40 individuals rules out such a possibility for the TG and PG phenotypes in regard to HLA (ABC), even with the two frequent supertypic specificities w4 and w6, which split the HLA-B antigens into two main groups<sup>18</sup>. It could be more informative to study the associations with the D polymorphism (mixed leukocyte reaction) and Da (D-locus associated) antigens, which are the equivalent of Ia antigens in the mouse.

### Discussion

Our results show that human peripheral blood lymphocytes carry acceptor sites for the mouse T cell factors for the synthetic polypeptides (T,G)-A-L and (Phe,G)-A-L. The abilities of being a 'high absorber' or a 'low absorber' are individual stable characters, which are inherited as simple Mendelian traits linked to the HLA

**Fig. 2** Absorption of mouse T-cell factors in a pedigree containing a recombination in the HLA complex. Abbreviations as in Fig. 1. a, b, c, and d represent the HLA haplotypes of the family members, c × d being the recombinant haplotype.



HLA haplotypes			Absorption loci		
	B	C	A	<i>p</i>	<i>t</i>
(a)	8	—	1	—	—
(b)	7	—	w24	+	+
(c)	w15	w3	3	—	+
(d)	7	—	2	+	—
(c × d)	7	—	3	+	+

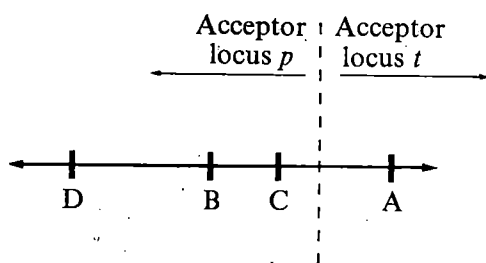


Fig. 3 HLA complex indicating preliminary mapping of the two acceptor loci *p* and *t*. *p*, acceptor locus for (Phe,G)-A-L factor, *t*, acceptor locus for (T,G)-A-L factor.

complex. This situation closely resembles that found in the mouse, where acceptor genes have been demonstrated in the I-region of H-2 and are good candidates for the Ir genes of that species. We therefore propose as a working hypothesis that the acceptor sites detected on human lymphocytes by these methods are products of the Ir genes of man and that high and low absorptions are equivalent to high or low immune responsiveness. To support this proposition, we must test the responsiveness of each individual to the antigen in question. Culture conditions have recently been established for the development of antibody responses by human lymphocytes, in which human PBL are stimulated with antigen and mouse T cell factor *in vitro*<sup>14,15</sup>. Thus, the correlation of factor absorption with responsiveness can now be tested.

The results described with HLA-recombinant families indicate the existence of at least two acceptor loci separable by crossing over. In the mouse, the acceptors are I-region products and carry the Ia antigenic determinants found predominantly on B lymphocytes<sup>7</sup>. In man, at least two loci in HLA have been shown to code for Ia-like B cell alloantigens, one associated with or identical to the HLA-D locus while the other is associated with the HLA-A locus<sup>19</sup>. A similar situation seems to exist in the Rhesus monkey<sup>20</sup>. There are also two loci determining the antigens stimulating the mixed lymphocyte reaction, another property of Ia antigens, with similar mapping<sup>21,22</sup>. In addition, disease associations suggest that some Ir genes may be closely linked to the HLA-D locus<sup>3</sup>. Therefore, on the basis of the detection of lymphocyte acceptor

sites described here, we propose the existence of two Ir gene loci (or regions) in the HLA complex and suggest that one of these is likely to be associated with HLA-D and the other with HLA-A. Further mapping studies are in progress to test this model. The existence of two Ir gene regions in the HLA complex could provide the basis for a selective advantage of certain haplotypes and hence lead to the linkage disequilibrium which is a feature of the distribution of HLA alleles in the population.

These *in vitro* tests of absorption and response may make it possible to study in detail the human Ir genes, avoiding the problems of *in vivo* immunisations of human volunteers with specific antigens of scientific interest. Moreover, the use of antigens and corresponding T factors of direct medical interest, could lead to assessment of predisposition to the HLA-associated diseases (for example, multiple sclerosis, gluten enteropathy), with a higher efficiency than by the present indirect Ir typing through the HLA (A B C D) markers.

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- Benacerraf, B. & McDevitt, H. O. *Science* **175**, 273 (1972).
- Sveigaard, A. *et al.* *The HLA System* (S. Karger, Basel, 1975).
- Dausset, J. & Sveigaard, A. (eds) *HLA and Disease* (Munksgaard, Copenhagen, 1977).
- Levine, B. B., Stember, R. H. & Fotino, M. *Science* **178**, 1201 (1973).
- Marsh, D. G., Bias, W. B. & Hsu, S. H. *Science* **179**, 691 (1973).
- Blumenthal, M. N., Amos, D. B., Noreen, H., Mendell, N. R. & Yunis, E. T. *Science* **184**, 1301 (1974).
- Munro, A. J. & Taussig, M. J. *Nature* **256**, 103 (1975).
- Taniguchi, M., Tada, T. & Tokuhisa, T. *J. exp. Med.* **144**, 20 (1976).
- Taussig, M. J. *Nature* **248**, 234 (1974).
- Mozes, E. in *The Role of Products of the Histocompatibility Gene Complex in Immune Responses* (eds Katz, D. H. & Benacerraf, B.) 485 (Academic, New York and London, 1976).
- Takemori, T. & Tada, T. *J. exp. Med.* **142**, 1241 (1975).
- Kapp, J. A., Pierce, C. W. & Benacerraf, B. in *The Role of Products of the Histocompatibility Gene Complex in Immune Responses* (eds Katz, D. H. & Benacerraf, B.) 569 (Academic, New York and London, 1976).
- Taussig, M. J., Munro, A. J., Campbell, R., David, C. S. & Staines, N. A. *J. exp. Med.* **142**, 694 (1975).
- Taussig, M. J., Munro, A. J. & Luzzati, A. L. in *The Role of Products of the Histocompatibility Gene Complex in Immune Responses* (eds Katz, D. H. & Benacerraf, B.) 553 (Academic, New York and London, 1976).
- Luzzati, A. L., Taussig, M. J., Meo, T. & Pernis, B. *J. exp. Med.* **144**, 573 (1976).
- Boyum, A. *Scand. J. Clin. Lab. Invest.* **21**, Suppl. 97 (1968).
- Cepellini, R., Curtioni, E. S., Mattiuz, P. L., Miggiano, V., Scudeller, G. & Serra, A. in *Histocompatibility Testing 1967* (eds Curtioni, E. S., Mattiuz, P. L. & Tosi, R. M.) 149 (Munksgaard, Copenhagen, 1967).
- Van Rood, J. J., Van Leeuwen, A. & Zweems, R. in *Histocompatibility Testing 1970*, (ed. Terasaki, P. I.) 93 (Munksgaard, Copenhagen, 1970).
- Mann, D. L., Abelson, L., Harris, S. & Amos, D. B. *Nature* **259**, 145 (1976).
- Balner, H. *Transpl. Proc.* **IX**, 837 (1977).
- Eijssvoegel, V. P., Van Rood, J. J., du Toit, E. D. & Schelekens, P. T. A. *Eur. J. Immun.* **2**, 413 (1972).
- Suci-Foca, N. & Rubinstein, P. *Transpl. Proc.* **IX**, 385 (1977).

## letters to nature

### Temporal and spectral variation of Cyg X-1

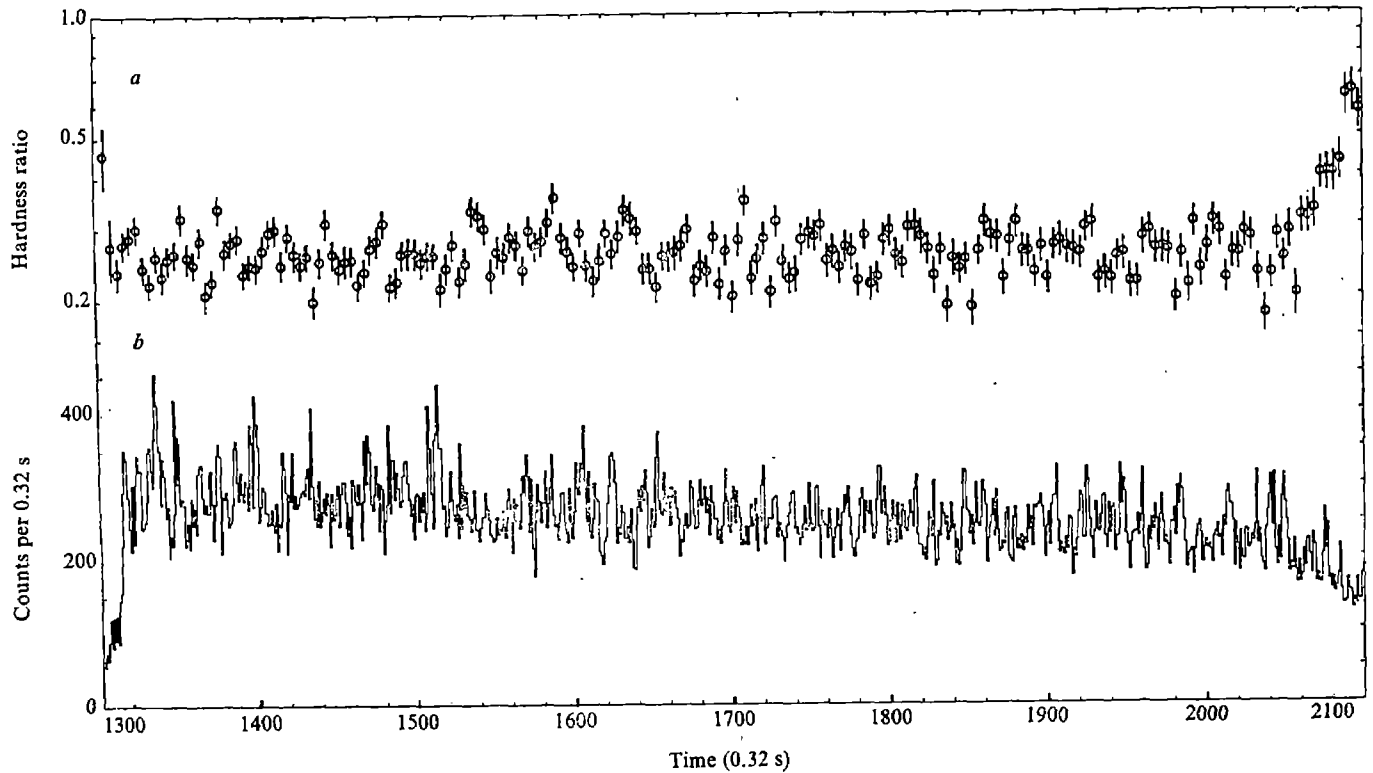
AMONG galactic X-ray sources, Cyg X-1 is distinctive because of its marked variability. Its X-ray intensity curve exhibits apparently aperiodic variation of large amplitudes<sup>1-3</sup>. It is tempting to suppose that this peculiarity of Cyg X-1 is related to the physical properties of the accretion disk formed around the black hole presumed to accompany the supergiant HDE226868 optically identified with Cyg X-1. We report here certain features of the subsecond variability concluded from a recent rocket observation.

The rocket observation of Cyg X-1 was carried out, using a rocket launched from Kagoshima Space Center, for ~250 s starting at 0502 UT 24 September, 1975 when the source was in the low-state. The total effective area of four Xe-CO<sub>2</sub> filled proportional counters was 680 cm<sup>2</sup>. The counts in every 0.625 ms time bin in three energy bands, nominally 1.5-5, 5-10 and 10-25 keV, hereafter referred to as L, M and H band respectively were telemetered. The counting-rate is corrected

for the small jittering of the rocket attitude.

The observed X-ray intensity profile is shown in Fig. 1 as a histogram of counts per time bin with a bin width of 0.32 s as an example. The intensity decreased by about 20% during the period of observation and in the first half period flare-like variations are more pronounced compared to the second half period.

The hardness ratio or spectral hardness averaged over 1.28 s, defined as the ratio of counting-rates of the ≤ 10 keV (L+M) and > 10 keV (H) bands, is also shown. It is clear that the spectral hardness also fluctuates in a complex manner with weak correlation to intensity variations, if there is any. The spectral variation becomes even more pronounced for shorter integration times of the order of 0.1 s. Correlation among counting-rate profiles of three energy bands corresponding to the spectral variation was investigated. It should be noted that the H-component occasionally fluctuates with characteristic time scales of the order of 0.1-1 s in a different



**Fig. 1** Intensity and spectral time profiles corrected for aspect are shown from the acquisition of the source until the re-entry of the rocket into the atmosphere. Time resolutions for the intensity and spectral hardness ratio are 0.32 s and 1.28 s respectively. In the first half of the observation, the intensity is approximately 20% higher and the flare-like activity is stronger compared to the latter half. The time scale is shown in units of 0.32 s. *a*, 10–25 keV/1.5–10 keV; *b*, 1.5–25 keV.

fashion from the L- and M-components resulting in the spectral variation.

We define the variability coefficient,  $\eta(t_b)$ , as the square root of the variance,  $V(t_b)_{\text{cyg}}$  due to intrinsic variation of the source, normalised to the average intensity,  $x(t_b)_{\text{cyg}}$ , where  $t_b$  is a bin width;

$$\eta(t_b) = \frac{(V(t_b)_{\text{cyg}})^{1/2}}{x(t_b)_{\text{cyg}}} \quad (1)$$

Statistical estimate of  $\eta(t_b)$  is

$$\eta(t_b) = \frac{(V(t_b) - x(t_b))^2}{x(t_b) - y(t_b)} \quad (2)$$

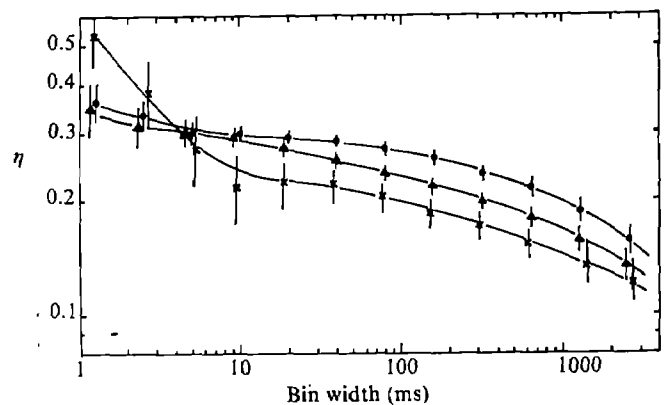
where  $V(t_b)$  is the total variance,  $x(t_b)$  is the average counts per bin and  $y(t_b)$  is the average background counts per bin;  $\eta(t_b)$ , which decreases monotonically with the increase of  $t_b$ , should exhibit a significant slope over a time range corresponding to the characteristic time scale involved in the variability. Figure 2 shows the variability coefficients as a function of the bin width for respective energy bands. The curves indicate the presence of a characteristic time scale from 0.1 to 1 s for all bands and, in addition, a time scale  $\leq 10$  ms for the H band. The 0.1–1 s time scale is also prominent in the autocorrelation function produced for this observation. The autocorrelation function is similar to that for observations already reported<sup>2,4</sup>. The presence of the  $\sim 10$  ms characteristic time scale was also notified by Canizares and Oda<sup>5</sup>.

In addition to the general variability, Cyg X-1 occasionally exhibits sporadic flares or bursts more than twice the average intensity and of the time scale of 1 s or a fraction thereof. The

sporadic flares from 0.1 to 1 s duration in general seem to have internal spectral structures, average spectral hardness being similar to the overall hardness: spectral softening in the flare is suggested.

It was demonstrated by Terrell<sup>6</sup> that the intensity time profile and the autocorrelation function of Cyg X-1 may be reproduced by the superposition of a number of random pulses of basically constant size and decay time in analogy to the shot-noise. The idea is that the variability is caused by the statistical fluctuation of the rate of underlying unit shot. If we take the shot-noise model with impulsive shots of the decay time  $\tau/2$  with the average shot rate  $\lambda \text{ s}^{-1}$ , the  $\eta$  against  $t_b$  curve approaches a constant  $f/(\lambda\tau)^{1/2}$  for  $\tau \leq t_b$  and tends

**Fig. 2** The variability coefficients against integration time for three energy bands are shown. A characteristic time scale of the order of a second or less is indicated. A  $\leq 10$  ms time scale is noted for the H-band. ●, h (1.5–5 keV); ▲, M (5–10 keV); XH (10–25 keV).





to decrease asymptotically as  $f/(\lambda t_b)^{1/2}$  for  $\tau \leq t_b$  where  $f$  is the fraction of the shot-noise component. Thus, from Fig. 2 we estimate  $\tau/2$  as 0.3 s and  $\lambda$  as  $20\text{ s}^{-1}$ , if  $f = 1$ , for the average intensity of the source at the low-state. In Fig. 2 the variance over the time range of 0.1–1 s is shown to be smaller for higher energies (H band) suggesting that  $\lambda$  is larger or  $f$  is smaller compared to the low energy band.

There are several features of the variability which have to be carefully investigated to see whether they resemble a simple shot-noise model with shot pulses of a constant decay time and size. First, the spectral variation shown by the time profile of the hardness ratio in Fig. 1 cannot be created by a single kind of the shot unless we assume an involved spectral and temporal internal structure of the shot. Second, to explain the difference of  $\eta$  for different energy bands instead of a single kind of shots we have to introduce an artificial ensemble of shots with the variety of spectral hardness: hard shots should be more abundant and smaller in amplitude to reconcile the above results, if  $f = 1$  as generally assumed, and  $\tau$  is constant. If we assume  $f < 1$  as suggested by Sutherland *et al.*<sup>7</sup>, the shot-noise model based on the constant shot can be used. If this were the case smaller  $f$  has to be assigned for higher energies, and a harder spectrum for the steady component compared to the shot-noise component and a negative correlation between the intensity and the hardness ratio are expected: the negative correlation, however, is not clear from this data. Third, the prominent flares can not be accounted for using the shot-noise model. In this experiment the estimated shot rate is about  $20\text{ s}^{-1}$ , if  $f = 1$ , and the model predicts a rapidly decreasing number of the flares with increasing amplitudes. In contrast, the number of the large flares observed is much more than predicted at least for the case of  $f = 1$ . For the case of  $f < 1$ , more complex arguments are necessary concerning the shapes of the large flares and the shots. On one occasion in October 1976 large flares were observed which could not be accounted for by the superposition of the shots regardless of assumed value of  $f$  (ref. 5).

While the shot-noise model is convenient to express the variability features in terms of the parameters and is also an attractive model providing physical implications as for the turbulent nature of the accretion disk, further studies seem necessary before the basic concept of the shot-noise model is firmly established.

Figure 2 indicates the presence of an additional characteristic time scale of  $\lesssim 10\text{ ms}$  for the H component. This is interpreted in terms of the existence of significant pulses of this time scale which will be discussed elsewhere.

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1. Oda, M. *et al. Astrophys. J. Lett.* **166**, L1–L7 (1971).
2. Boldt, E., Holt, S., Rothschild, R. & Serlemitsos, P. *Proc. Calgary Conf. X-rays in Space* 69–127 (1974).
3. Oda, M., Doi, K., Ogawara, Y., Takagishi, K. & Wada, M. *Astrophys. Space Sci.* **42**, 223–244 (1976).
4. Weisskopf, M. C., Kahn, S. M. & Sutherland, P. G. *Astrophys. J. Lett.* **199**, L147–L151 (1975).
5. Canizares, C. R. & Oda, M. *Astrophys. J. Lett.* **214**, L119–L122 (1977).
6. Terrell, N. J. *Astrophys. J. Lett.* **174**, L35–L41 (1972).
7. Sutherland, P. C., Weisskopf, M. C. & Kahn, S. M. *Columbia Astrophys. Lab. Contr.* No.136 (1977).

## Temperature measurement of interplanetary–interstellar hydrogen

PHOTOMETRIC observations can be used to measure the velocity of the Solar System through an interstellar medium and also provide an accurate method of measuring the temperature of that medium, provided that a very narrow filter is used in order to determine the emission linewidth. We present here the results obtained using a hydrogen absorption cell in conjunction with a Lyman- $\alpha$  photometer contained in the Soviet scientific spacecraft Prognoz-5 which was launched in November 1976. We use only the  $\text{La}$  results to measure the temperature of the interplanetary–interstellar hydrogen. The high result we obtained indicates that the Solar System may be moving through an intercloud medium heated by cosmic- or soft X-rays.

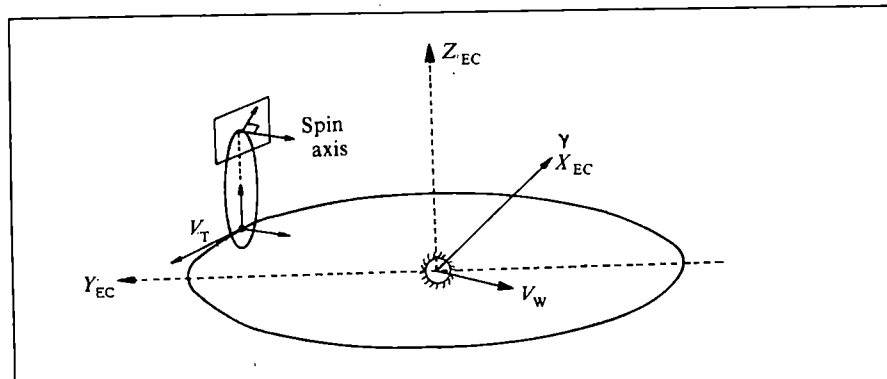
The motion of the Solar System relative to the nearby stars is characterised by a velocity vector  $V$  of  $20\text{ kms}^{-1}$  in the Apex direction,  $\alpha = 271^\circ$  and  $\delta = 30^\circ$ . In this motion, the Solar System is swept by neutral matter of the local interstellar medium. In particular, the flow of interstellar hydrogen atoms was first observed<sup>1,2</sup> through resonance scattering of solar  $\text{La}$  photons (121.6 nm). Helium atoms were also later observed<sup>3</sup> (at 58.4 nm). These observations confirmed the prediction that hydrogen and helium atoms can penetrate deeply into the solar system, because the heliosphere boundary is presumably quite transparent to neutral matter. From the photometric observations at 121.6 and 58.4 nm, it was found that the Solar System motion through the interstellar medium is characterised by a vector  $V_w$ , pointing to a direction  $\alpha = 252^\circ$ ,  $\delta = -15^\circ$ , differing from the Apex direction by  $\approx 50^\circ$ . The density<sup>4</sup> of hydrogen was measured in the range  $0.08\text{--}0.12\text{ atom per cm}^3$  while for helium<sup>3</sup> it was found to be in the range  $0.009\text{--}0.024\text{ atom per cm}^3$ . An estimate of the temperature can also be derived from photometric observation but with considerable uncertainty:  $10^3\text{--}15 \times 10^3\text{ K}$  from  $\text{La}$  photometric observations<sup>4,5</sup>,  $2.5 \times 10^3\text{--}10^4\text{ K}$  from helium observations<sup>3</sup>.

A much more accurate method to determine the temperature is to measure the line width of solar  $\text{La}$  scattered by hydrogen atoms. One attempt was made with the help of *Copernicus* high resolution spectrometer<sup>6</sup>, with a result lying in the range of  $5\text{--}20 \times 10^3\text{ K}$ , rather wide because of the poor photometric sensitivity. Another measurement was obtained<sup>7</sup> from a hydrogen absorption cell placed on board the Soviet probe Mars 7. Though the temperature range was narrowed to  $6\text{--}13 \times 10^3\text{ K}$ , it was still rather large, because of poor knowledge of the direction of sight. The favoured value was  $12 \times 10^3\text{ K}$ .

Results obtained recently with the same technique (a hydrogen absorption cell associated with a  $\text{La}$  photometer) yielded a much more accurate measurement of  $8.8 \pm 1 \times 10^3\text{ K}$  for the temperature of the nearby interstellar medium. The scientific Soviet spacecraft Prognoz 5 was launched on 25 November, 1976 on a very eccentric orbit, with an apogee distance to the centre of the Earth of  $203 \times 10^3\text{ km}$ , and a period of nearly 4d. The spin axis of the spacecraft was pointed toward the sun, and the spin rate was  $3^\circ$  per s. One of the instruments placed on board was a four channel ultraviolet photometer for the study of hydrogen and helium in the interplanetary medium and in the exosphere, through the observation of resonance scattering of solar light at 121.6 nm (H,  $\text{La}$ ), 58.4 nm (He), and 30.4 nm ( $\text{He}^+$ ). The  $\text{La}$  channel looked at  $90^\circ$  from the spin axis, with a field of view of  $1.1 \times 2.7^\circ$  and a 1-s integration time of photon pulses. An absorption cell was placed in front of the photomultiplier.

The apogee of the spacecraft as observed from Earth was nearly in the direction of the North Ecliptic Pole (Fig. 1). The  $\text{La}$  intensity, recorded around the apogee and measured in the plane of rotation of the spacecraft as a function of roll angle  $\phi$ , is shown on Fig. 2. The Earth and its hydrogen geocorona is

**Fig. 1** Configuration of orbit of Prognoz-5 and its spin axis in an ecliptic coordinates system. On 1 December 1976, the Earth was nearly opposite to the direction of  $V_w$ , the velocity of the sun relative to the nearby interstellar medium.

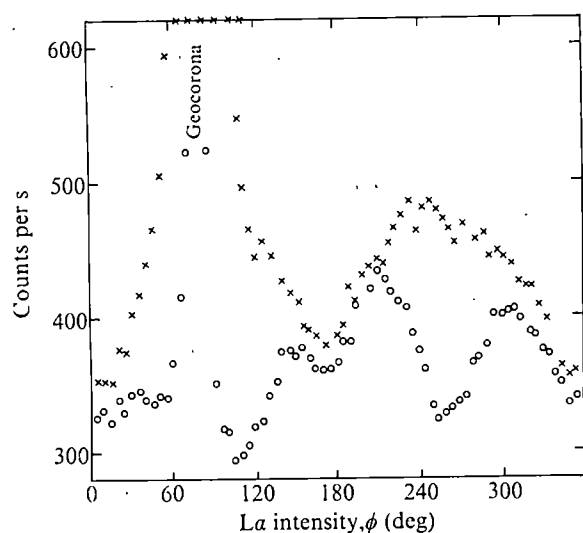


clearly seen from  $\phi = 0^\circ$  to  $\phi = 170^\circ$ , whereas the interplanetary  $L\alpha$  emission is observed from  $\phi = 170^\circ$  to  $\phi = 360^\circ$ , free of any geocoronal contamination.

The absorption cell can be electrically activated, with an optical thickness  $\tau = 8$  and a temperature  $T_c = 300$  K. Thus all of the emission within  $\pm 1.4 \times 10^{-3}$  nm of the  $L\alpha$  wavelength  $\lambda_0 = 121.566$  nm is blocked, and the measured intensity is decreased by a factor  $R$ , the reduction factor. The absorption of the hydrogen cell is large on the geocorona ( $R$  is small) because the geocoronal linewidth is small, whereas the absorption is small ( $R$  is large) on the interplanetary background.

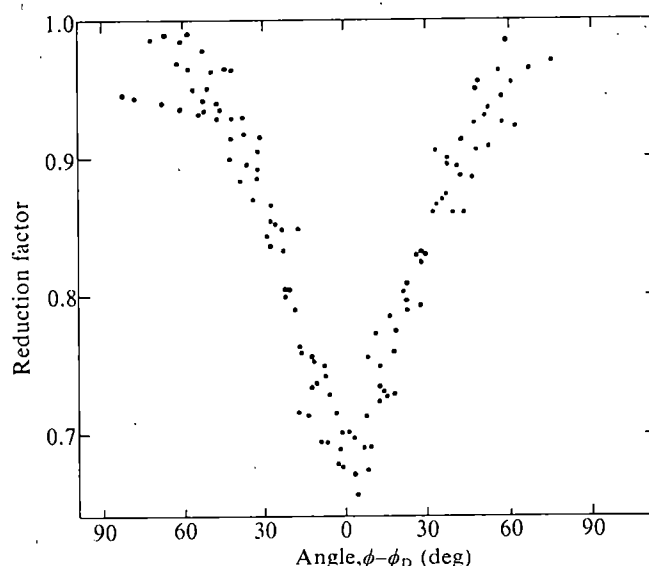
For a pure gaussian emission profile, the reduction factor is only a function of the temperature of emission,  $T$  and of the Doppler shift velocity  $V_D$  between the instrument and the emitting medium. This function can be accurately computed<sup>7</sup> when the hydrogen cell parameters,  $\tau$  and  $T_c$  are known. At apogee, the spacecraft velocity with respect to the Earth is negligible, whereas the Earth's velocity  $V_T$  on its orbit around the Sun is  $30 \text{ km s}^{-1}$  (Fig. 1); therefore there is a strong modulation of Doppler shift  $V_D$  as a function of direction of sight, defined by the roll angle  $\phi$ , the origin of which lies in the ecliptic plane (Fig. 2). We interpret the strong dip of  $R$  around  $\phi_D = 260^\circ$  as a consequence of this modulation.

The observations were made on 1 December 1976, when the spacecraft was practically 'downwind'; therefore the modulation of Doppler shift is essentially caused by  $V_T$  and not by  $V_w$  whose projection on observation plane should be small. We



**Fig. 2** The  $L\alpha$  intensities are plotted as a function of roll angle,  $\phi$  with and without the hydrogen-cell activated.  $\times$ , Cell off;  $\circ$ , cell on. The ecliptic plane lies at  $\phi = 0^\circ$  and  $\phi = 180^\circ$ . The most intense part of the geocorona is not shown. The interplanetary background is clearly seen from  $\phi = 180^\circ$  to  $\phi = 360^\circ$ , opposite to the geocorona. The absorption cell is most efficient at  $\phi_D \approx 260^\circ$ , where the Doppler shift is zero.

assume that the velocity distribution of hydrogen atoms is not perturbed by the sun; therefore at each point of the solar system the emission profile is a pure Doppler profile characterised by  $T$ . The motion of the spacecraft through the interplanetary matter is  $V_{rel} = V_T + V_w$ . When the direction of sight is perpendicular to  $V_{rel}$ , the Doppler shift is zero and the temperature,  $T$ , is directly derived from the measured value of  $R$ . This should occur twice along the great circle of observation, and at these points,  $R$  should be minimum. One point is identified at the angle  $\phi_D = 260^\circ$  where the minimum value  $R_{min}$  is measured, the other is somewhat occulted by the geocorona. In Fig. 3 all the measurements of  $R$  recorded for 2 d



**Fig. 3** All the measurements of the reduction factor,  $R$ , collected during two days are plotted as a function of  $\phi - \phi_D$ . A minimum value of  $R = 0.68 \pm 0.01$  is deduced from this graph, yielding the temperature of the nearby interstellar medium,  $T = 8,800 \pm 1,000$  K.

in the region around  $\phi_D = 260^\circ$  were plotted, yielding a measured value of  $R_{min} = 0.68 \pm 0.01$  from which is derived directly the temperature  $T = 8.8 \pm 1 \times 10^3$  K (taking into account an uncertainty of 10% on  $\tau$ ). This determination, though in the range  $6-13 \times 10^3$  K found with the same technique with Mars 7 is somewhat lower than the favoured value  $12 \times 10^3$  K. But the photometric quality of the present experiment is so much better that we favour this value of  $T = 8.8 \pm 1 \times 10^3$  K.

Such a high temperature of the nearby interstellar medium, associated with the low density of  $\approx 0.1$  atom per  $\text{cm}^3$ , strongly suggest that the Solar System is moving through the so-called

'intercloud medium' (ICM) and confirm the validity of a hot ICM (ref. 8), heated by low-energy cosmic rays or by soft X rays<sup>9,10</sup>.

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1. Bertaux, J. L. & Blamont, J. E. *Astr. Astrophys.* 11, 200 (1971).
2. Thomas, G. E. & Krassa, R. F. *Astr. Astrophys.* 11, 218 (1971).
3. Weller, C. S. & Meier, R. R. *Astrophys. J.* 193, 471 (1974).
4. Bertaux, J. L., Ammar, A. & Blamont, J. E. *Space Research* 12, 1569 (Akademie-Verlag, Berlin, 1972).
5. Thomas, G. E. *The Solar Wind* (eds Sonnett, Coleman & Wilcox) 668 (NASA SP-308, 1972).
6. Adams, T. F. & Frisch, P. C. *Astrophys. J.* 212, 300 (1977).
7. Bertaux, J. L. *et al. Astr. Astrophys.* 46, 19 (1976).
8. Field, G. B., Goldsmith, D. W. & Habing, H. J. *Astrophys. J.* 155, L 149 (1969).
9. Silk, J. & Werner, M. *Astrophys. J.* 158, 185 (1971).
10. Dalgarno, A. & Mc Cray, R. A. *A. Rev. Astr. Astrophys.* 10, 375 (1972).

## Absence of detectable X rays from supercluster candidate 4U0134-11

A CORRELATION between a small subset of high galactic latitude X-ray sources and superclusters of galaxies has been suggested<sup>1</sup>. The existence of superclusters (that is, clusters of clusters of galaxies) was first discussed in the context of optical observations<sup>2,3</sup>. A hot intrasupercluster gas could lead to detectable X-ray emission as well as provide a large fraction of the mass necessary to close the universe<sup>4</sup>. The Goddard Space Flight Center Cosmic X-ray Spectroscopy experiment onboard Orbiting Solar Observatory 8 (OSO 8) scanned directly over the position of 4U0134-11, one of the three 4U sources associated with a supercluster. We did not observe X rays from this source with an upper limit to the source strength a factor of 6 below the catalogued Fourth Uhuru (4U) strength.

Figure 1 represents the path of the OSO 8 spin axis through the region of 4U0134-11. The observation was performed between 7 and 13 July 1976. Catalogued X-ray sources in this region and their error boxes are shown. The detector is a xenon-filled proportional counter, efficient from

Fig. 1 The OSO 8 spin axis scan path during the observation of 4U0134-11. The known X-ray sources and their error boxes are shown. Portions of the scan path used to determine the background rate extend out of the picture.

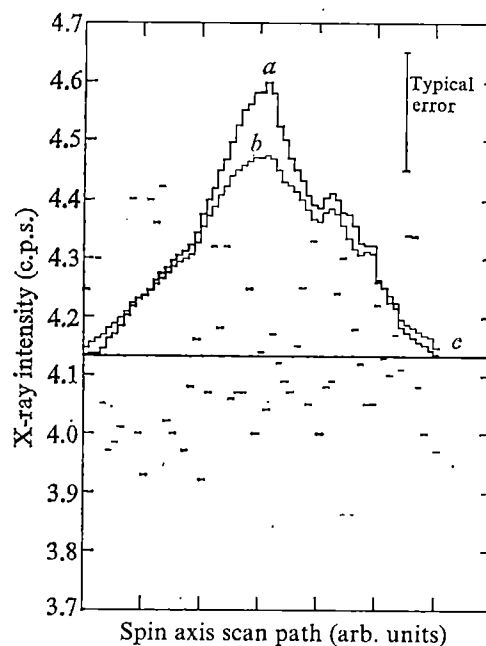
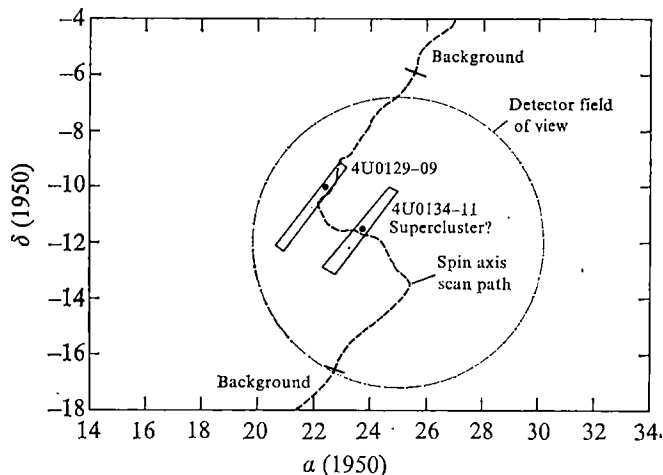


Fig. 2 Models of the X-ray intensity during the scan path superimposed on the data. Reduced  $\chi^2$  are a, point source 5.1; b, diffuse source, 4.2; c, no source, 1.3.

2 to 60 keV, with an effective area of 237 cm<sup>2</sup> (ref. 5). It is pointed along the spin axis and has a 5° FWHM field of view. The background rate was deduced from the rates at source-free adjacent regions.

The counting rates along the scan path were best fit by a model with a constant X-ray background and no sources. The data (and typical error) are shown in Fig. 2. The three histograms superimposed on the data are: (1) a model with 4U0134-11 as a point source at 2.1 Uhuru counts, the lower statistical limit of its 4U strength; (2) a model with 4U0134-11 as a 2° in radius diffuse source with the same strength; and (3) a model without sources. For each model the deduced X-ray background is added to the count rate and to obtain an upper limit for the strength of 4U0134-11, the strength of 4U0134-09 is set equal to zero. The reduced  $\chi^2$  for the models are 5.1, 4.2, and 1.3 respectively. These results are not sensitive to the absolute level of the background. That is, the no-source model is always by far the best fit. We obtain an upper limit at 99% confidence of 0.4 Uhuru counts for an extended (2° in radius) source at the position of 4U0134-11 (4U value =  $2.7 \pm 0.6$  counts). Because of our large field of view and the motion of our spin axis directly over the source positions, we are not sensitive to positional changes of the sources within their 4U error boxes. Our upper limit at 99% confidence for the sum of the intensities of 4U0129-09 and 4U0134-11 is also 0.4 Uhuru counts.

The X-ray spectrum associated with the supercluster candidates is consistent with a thermal bremsstrahlung spectrum with temperature of more than 10<sup>8</sup> K (ref. 6). Therefore, the fact that the Uhuru detectors<sup>7</sup> are efficient to slightly lower X-ray energies than this OSO 8 detector (1.7 keV rather than 2 keV) does not affect our relative sensitivity to this hard spectrum.

Three high galactic latitude X-ray sources have been identified with superclusters<sup>4,8</sup>. Our observations indicate that one of these, 4U0134-11, is either variable or spurious. Other observations<sup>8</sup> with this detector indicate that it can detect X-ray sources with strengths less than the nominal 4U strength for this object and determine their spectra with comparable observing time. Variability of the X rays from 4U0134-11 on a time scale of ~ years, implying a source

diameter of  $\sim 1$  pc, would rule out a supercluster identification. We have not yet performed observations of the other 4U supercluster candidates.

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1. Forman, W. *et al. Astrophys. J. Suppl.* (in the press).
2. Abell, G. O. *Astrophys. J. Suppl.* no 21, 3, 31 (1958).
3. Abell, G. O. *Astr. J.* 66, 601 (1961).
4. *Sky and Telescope* 54, 105 (1977).
5. Pravdo, S. H. *et al. Astrophys. J. Lett.* 206, L41 (1976).
6. Murray, S., Giacconi, R., Forman, W., & Jones, C., *Astrophys. J.* (submitted).
7. Giacconi, R., Kellogg, E., Gorenstein, P., Gursky, H. & Tananbaum, H. *Astrophys. J. Lett.* 165, L27 (1971).
8. Mushotzky, R. F., Serlemitsos, P. J., Smith, B. W., Boldt, E. A. & Holt, S. S. *Astrophys. J.* (submitted).

## Is the Sun a pulsar?

WHILE investigating intensity variations of spectrum-lines of solar origin, periods which seem to describe the Sun's oscillation have been found. The observed period of 2.65 h is very close to 2.78 h, the predicted radial mode fundamental oscillation of the homogeneous model of the Sun. Thus some observations of the solar disk seem to confirm the existence of a radial mode pulsation, while others give no significant indication of such a period. External effects may not, however, have been filtered satisfactorily in all cases. Thus we tried to find an effect of magnetospheric origin that can be related in some way to the described observations. We report here observations of pulsations with period of 2 h 40 min.

Fluctuations of solar origin having a stable period have been noted independently by various observatories<sup>1,2</sup>. From their measurements a characteristic period of 2 h 40 min can be assigned to fluctuations of the central parts of the solar disk. It was established that this event is independent of local time and shows no correlation to the starting time of observation. It is thought that the phenomenon is not due to the apparatus, but originates from the Sun. The observed oscillations can be interpreted as the Sun's pulsations, or perhaps alien effects originating perhaps from the magnetosphere and atmosphere.

The magnetosphere of the Earth can be regarded as a very sensitive measuring probe in the solar wind, which senses every perturbation of solar origin. Thus we investigated variations of the Earth's magnetic field from the viewpoint of effects caused by pulsations of solar origin. There are several magnetic recording systems in the Tihany Observatory<sup>3,4</sup>. We used their data to select several periods to investigate pulsations within the range of periods indicated above.

Magnetically quiet cycles were not considered due to the relative height of local noise level. Records of great magnetic storms were useless as the variations are of large amplitudes and suppress other effects. Hence, we investigated magnetically active days with magnetic variations between 50 and 100 nT.

Diurnal sections from the data arrays of such days were individually analysed. This allowed us to eliminate phase

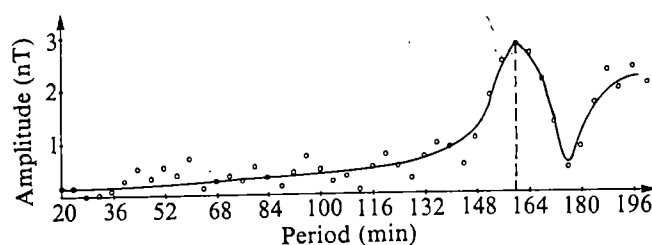


Fig. 1 Amplitude spectrum of the variations of the geomagnetic field for 10 November 1975 in the range of periods from 20 to 200 min.

springs which affected the analysis. Thus an amplitude spectrum characteristic of the diurnal part of the selected magnetically active days was obtained as shown in Fig. 1. An amplitude peak is seen to rise by a maximum of several nT out of the average noise level in the environment of the period 2 h 40 min. Most of the investigated days gave a similar result. In Fig. 2 is shown the mean spectrum for 9–11 November 1975. On several occasions no significant rising amplitude peak was found on the noise level background.

These results can be interpreted as follows. We assume that the period of 2 h 40 min is of solar origin, during magnetically active cycles the magnetosphere of the Earth and the magnetic lines of force of the interplanetary field intertwine.

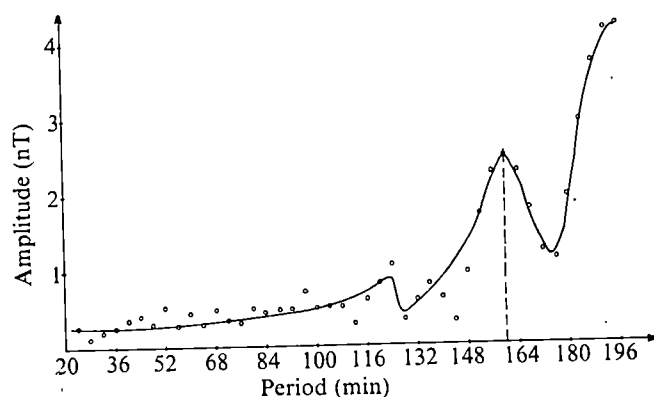
Such a connection means that magnetic field variations originating from the Sun's pulsation, which in the interplanetary space reaches as far as the magnetosphere, perturb the geomagnetic field. This can be observed on magnetic records in the observatories. Since the oscillation observed on the Sun's surface corresponds to magnetic variations of Gaussian magnitude, variations of several nT ( $10^{-5}$  G) amplitudes are possible on the Earth's surface.

During magnetically quiet cycles the magnetosphere is closed, there is no merging, and so no pulsation of solar origin can be observed. During magnetic storms, however, the noise level rises to such an extent that, although the merging exists, no significant amplitude could be detected around the above period.

We do not know whether spectrum lines investigated by the astrophysical observatories are insensitive to magnetic effects in a way that they are not affected by disturbances of the magnetosphere. If this were possible, then the possibility of a pulsation of a so far unknown terrestrial magnetospheric origin cannot be excluded. Hence the observed spectrum lines may be influenced by such a pulsation, which may have seemed to be of solar origin.

Thus, if the possibility that spectrum lines with 5,123.7 Å wavelength can be affected by geomagnetic field variations is excluded, then the magnetic variation with a period of 2 h 40 min

Fig. 2 Mean amplitude spectrum of the variations of the geomagnetic field for the cycle 9–11 November 1975.





originating from the magnetosphere presents an indirect proof of the Sun's oscillations. Thus the Sun with nearly homogeneous distribution of density has a radial mode fundamental oscillation within the range of the above period. At the same time this phenomenon may prove that the interplanetary and terrestrial magnetic field merge.

For a final conclusion to be reached, however, the observed spectrum line of solar origin must be examined for its sensitivity to variations of the geomagnetic field. If it is insensitive, the possibility of a magnetospheric effect can be excluded.

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1. Severny, A. B., Kotov, V. A. & Tsap, T. T. *Nature* 259, 87-89 (1976).
2. Brookes, J. R., Isaak, G. R. & van der Raay, H. B. *Nature* 259, 92-95 (1976).
3. *A. Rep. Hungar. Geophys. Inst.* 1975.
4. *A. Rep. Geophys. Observ. Tihany* 1975.

## Short-term influence of anthropogenic sources on tropospheric baseline lead

ESTIMATING tropospheric baseline lead requires relatively long collection periods using conventional high volume methods because the airborne concentrations are very low in comparison with levels found in urban areas. During such collections, anthropogenic sources of lead may be present for short periods leading to apparently high results, even at remote locations such as the mid-Pacific Ocean<sup>1</sup> and Antarctica<sup>2</sup>. Significant geographical variations in soil-sized Pb particles were also related to anthropogenic sources affecting the distribution of lead in marine atmospheric particulates<sup>3,4</sup>. We describe here investigations using furnace atomic absorption spectrometry (AAS) of the lead content in the atmosphere around Cape Grim, Tasmania.

High volume particulate collections give average results which do not account for atmospheric changes occurring over a few hours because of the collection times required. Limitations are also imposed by the sensitivity of the analytical technique for lead determination and the blank levels of filter media used. Furnace atomic absorption spectrometry (AAS) has been proposed as a suitable technique for the determination of background atmospheric lead particulates<sup>5</sup>, using the graphite furnace atomiser as the particulate collection device and taking advantage of the extremely high sensitivity for lead and small air sample volumes required. We have used graphite cups in the dual role of collection device and furnace atomiser, using micro air-sampling techniques based on earlier work<sup>6-8</sup> to follow atmospheric lead concentration over short periods of time, 7 h (but 3-10 min in urban areas) using a consecutive series of individual samples. The graphite cups (RW1 grade Ringsdorff Werke) gave the unique advantage of a filter with a zero blank for lead which can be transported to and from the sampling location without contamination<sup>9</sup>.

The site for sampling was the Australian Baseline Atmospheric Pollution Station (Department of Science) at Cape Grim, North West Tasmania, located in the Southern Hemisphere as shown in Fig. 1a. The station is situated on the edge of a cliff, 90 m above the sea and receives western maritime winds which travel over the Southern Ocean at this latitude. But winds travelling around high pressure centres (counter-clockwise air flow in the Southern Hemisphere) and within 90° of north east may be subjected to anthropogenic lead sources from urban-industrial sources in Tasmania and the mainland of Australia (Fig. 1b).

A series of 15 consecutive samples were taken over five days (Table 1 and Fig. 2). Other measurements taken on site, over the

same period, were a high volume sample (Table 1), meteorological data (Fig. 2) and a comparison of hygroscopic (sea-salt) particles and non-hygroscopic particles per unit volume (Table 2).

Meteorological data for the period 14-16 May (Fig. 1) showed a steady easterly wind (low wind speed) proceeding towards Cape Grim from the urban-industrial centres along the northern coastal area of Tasmania. This apparently has a strong influence on the total particle concentration (Table 2). Between 16 and 17 May the wind reversed direction (easterly to westerly). Sampling of lead particulates was started then.

Samples 1-4 showed very high atmospheric lead concentrations which seemed to be anomalous at first examination. The reversal of the steady air mass, however, from the previous few days was associated with precipitation (rainfall) on 16 and 17 May and caused the atmospheric lead concentrations to be as high as typical urban levels. The influence of precipitation is also apparent from the particle data (Table 2).

Following the cessation of precipitation (sample 4) and the displacement of all impure air, the westerly winds brought very clean maritime air to Cape Grim and the atmospheric lead concentration fell to very low levels (samples 5-12). The total particle concentrations (Table 2) from late on 17 May until the middle of 21 May were the lowest for the whole period.

On 20 May a wind change to north-north westerly direction led to an increase in atmospheric lead concentration (samples 13-15) but in this instance directly following the wind change, implicating the influence of anthropogenic sources. This was also

Table 1 Atmospheric lead particulate concentrations at Cape Grim

Sample no.	Date May 1976	Sampling period (h)	Sampling time (min)	Pb ( $\mu\text{g m}^{-3}$ )
1	17	0758-1500	422	0.086
2	17	1558-2258	420	0.109
3	18	0000-0700	420	0.213
4	18	0835-1535	420	0.043
5	18	1603-2300	417	0.0044
6	19	0006-0710	424	0.0084
7	19	0906-1605	419	0.0014
8	19	1653-2355	422	0.0033
9	20	0017-0717	420	0.0032
10	20	0922-1928	606	0.0037
11	20-21	1945-0043	298	0.0011
12	21	0107-0818	421	0.0009
13	21	0907-1609	422	0.015
14	21-22	1708-0013	423	0.019
15	22	0016-0718	422	0.040
Mean	Samples 1-15	$0.037 \pm 0.059 \mu\text{g m}^{-3}$ Pb		
Mean	Samples 5-12	$0.0033 \pm 0.0024 \mu\text{g m}^{-3}$ Pb (base line)		

Analysis for Pb furnace AAS was performed using the Varian Techtron Model 63 CRA following addition of  $2 \mu\text{l}$   $1,000 \mu\text{g ml}^{-1}$   $\text{H}_3\text{PO}_4$  to each cup and Pb standards in 20%  $\text{HNO}_3$  (double distilled). The Model 63 CRA workhead was mounted in a Model 1200 AAS which was set to the 217.0 nm Pb resonance line and 1.0 nm slit. Non-atomic absorption was negligible. Detection limit for 10-l air sample was  $0.0036 \mu\text{g m}^{-3}$  Pb and  $> 20 \text{ l}$  air was sampled for baseline conditions, identified from on-site meteorological equipment. Total volume of air passed through each cup with a Millipore Type XX60 220 50 pump, was measured using an American Dry Type Test Meter (Model DTM 115) because flow-rates through individual cups were variable (range  $0.028-0.31 \text{ l min}^{-1}$ ). During preparation each sample and blank cup was fired through the atomiser cycle for Pb to check that contamination was at blank level before placing in Teflon adapters and glass containers<sup>9</sup> and transportation to sampling site. Dimensions of RW1 grade graphite cups are as described for other cups<sup>8</sup>.

High volume air sample (Kimoto HVA and Whatman GF/A glass-fibre filter) 0804 h 17 May-0717 h 22 May  $0.0056 \pm 0.0011 \mu\text{g m}^{-3}$  Pb. Measurement of air flow-rate was made twice daily during the collection period and used to calculate the volume of air sampled ( $9,692 \text{ m}^3$ ). Whatman GF/A filter was divided into two and extracted with 10 ml 20%  $\text{HCl}$  and 40 ml 40%  $\text{HNO}_3$  (both high purity) in a Soxhlet extractor for 3 h. Solution was taken to near-dryness and made up with 10%  $\text{HNO}_3$  to 50 ml after filtering. Average of 4 filters, treated similarly, was taken for blank. Pb was determined with Pye-Unicam Model SP1950 AAS incorporating automatic background correction and gave a detection limit of  $0.0008 \mu\text{g m}^{-3}$  Pb (217.0 nm Pb resonance line), in this case.

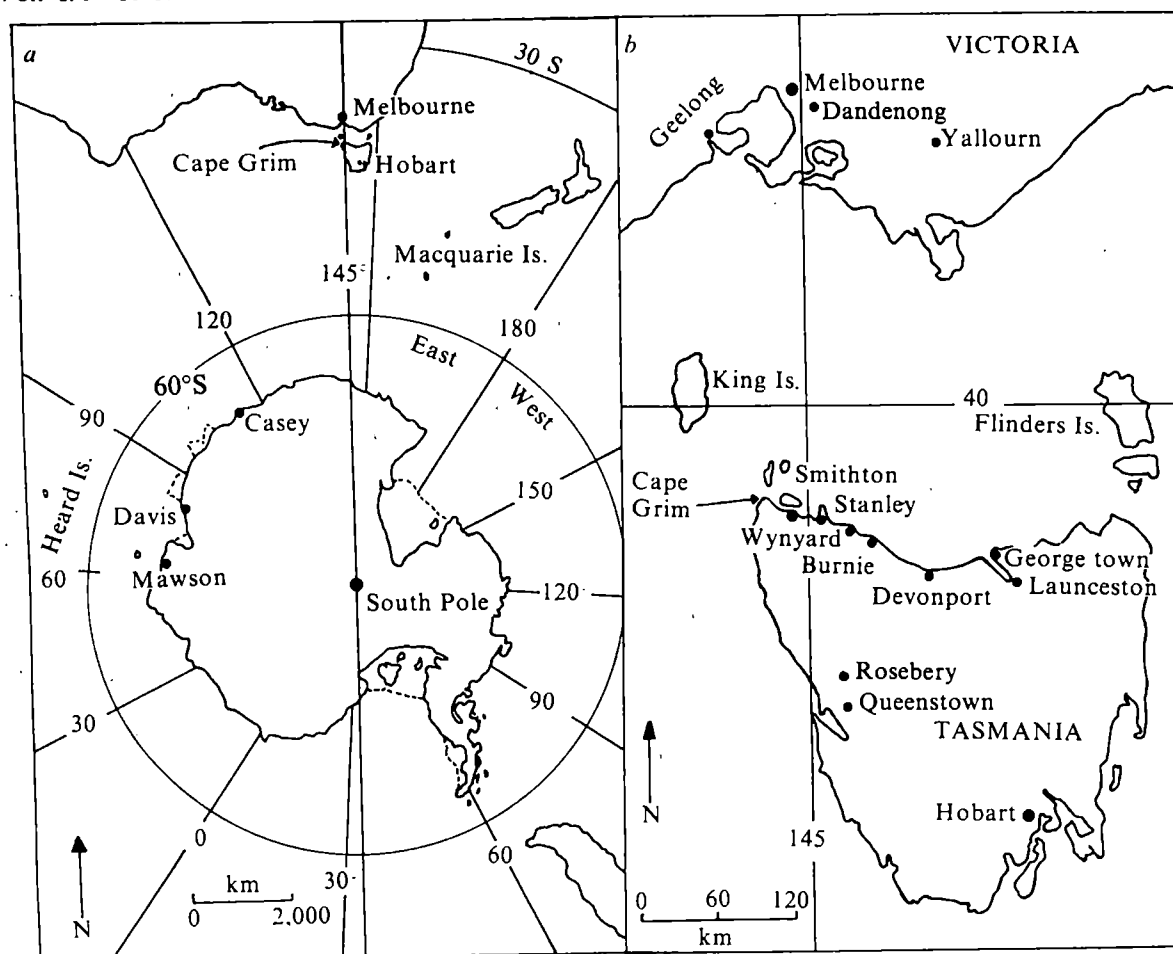


Fig. 1 Location of Cape Grim in relation to *a*, Southern Hemisphere and *b*, Urban and industrial centres of Tasmania and the mainland of Australia.

associated with a dramatic increase in total particle concentration (Table 2) compared with sea-salt particle concentration demonstrating the interaction of air from an urban origin with the background air and the importance of meteorological data in interpreting the atmospheric lead results.

The efficiency of graphite as a filtration medium may be judged by comparing the mean for samples 1–15 ( $0.037 \mu\text{m}^{-3}$  Pb) with the high volume sample result ( $0.0056 \mu\text{m}^{-3}$  Pb). Both figures include lead from anthropogenic and background origins and hence the high volume figure is not indicative of baseline atmospheric concentration. Glass-fibre filters as used in high volume samplers, are reported to be greater than 99% efficient for di-(2-ethylhexyl)phthalate particles ( $0.3 \mu\text{m}$  in diameter) at a flow rate of  $0.1 \text{ ml min}^{-1}$  (ref. 10). Comparison of graphite with common filter media, including glass-fibre for urban atmospheric lead particulates, showed graphite to be a much more efficient filter material, presumably because it retains a larger proportion of sub- $\mu\text{m}$  lead-containing particles<sup>11</sup>. Studies of size distributions of airborne particles at various urban and non-urban locations are almost always multi-modal<sup>12</sup> and considerable proportions of particles can be in the size range  $0.05$ – $1.0 \mu\text{m}$ . The size distribution of the smaller particles (Table 2) (including lead) has a mode between  $0.01$ – $0.05 \mu\text{m}$  diameter in polluted conditions and may be as great as  $0.1 \mu\text{m}$  or larger in clean conditions (E. K. Bigg, personal communication). Sea-salt particles (Table 2), by comparison, have a modal diameter of about  $1 \mu\text{m}$  and rarely have diameters below  $0.3 \mu\text{m}$ . This information supports the theory that the glass-fibre filter collection (Table 1) was not retaining a large proportion of sub- $\mu\text{m}$  lead-containing particles. Particles of the order of  $0.1 \mu\text{m}$  will have longer atmospheric residence times compared

with those less than  $0.01 \mu\text{m}$  or greater than  $10 \mu\text{m}$ , which tend to agglomerate, be washed out, or become 'fall-out'.

The mean figure for samples 5–15 ( $0.0033 \mu\text{m}^{-3}$  and equivalent to  $3.3 \text{ ng per SCM}$ ) describes the background atmospheric lead concentration at Cape Grim for the period sampled and compares in magnitude with data from the remote HASL Southern Hemisphere stations<sup>13</sup> (Easter Island, Antarctica (Chilean stations), South Pole station) for the period 1966–1976.

Table 2 Nature of air particles at Cape Grim (comparison of hygroscopic with non-hygroscopic particles)

Date	8-h collection beginning at denoted hour			
	0000 (M/N)*	0600 (M/N)	1200 (M/N)	1800 (M/N)
May 1976				
14	0.6/448	0.67/—	0.67/480	0.69/560
15	0.66/—	0.60/576	—/—	—/—
16	2.9/432	3.1/—	3.2/—	3.6/272
17	3.4/344	—/—	—/—	4.7/144
18	4.4/—	4.2/—	4.8/160	3.8/—
19	3.6/154	—/—	—/—	3.6/164
20	2.4/150	—/—	—/256	1.8/—
21	2.6/—	3.6/128	3.7/—	4.1/936
22	—/—	—/—	—/—	3.4/126

Lead particulates are associated with non-hygroscopic particles (difference of sea-salt and total particles) but particles from urban sources are very often mixed. Sea-salt particles form about 0.1% (numerically) of particles in polluted conditions and strictly non-hygroscopic particles; 0.1–10%. Most non-sea-salt particles usually have a hygroscopic coating (E. K. Bigg, personal communication).

\*M is salt particles  $\text{cm}^{-3}$  (hygroscopic) and N is total particles  $\text{cm}^{-3}$  (electrostatic precipitator) as weighted running mean over at least 24 h. (E. K. Bigg, personal communication.)

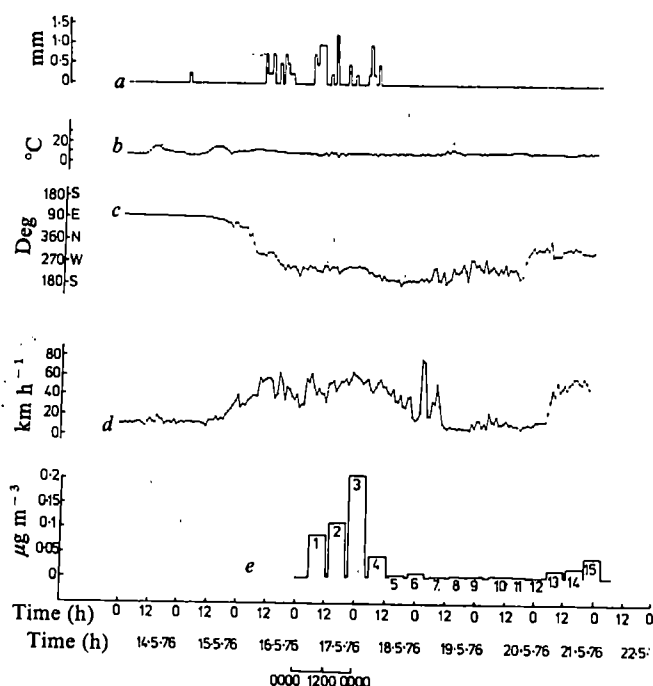


Fig. 2 Diurnal variations of atmospheric particulate lead, wind speed and direction, temperature and precipitation at Cape Grim. *a*, Precipitation for whole hour; *b*, temperature for minutes 6–9 of the hour; *c*, mean wind direction for minutes 3–6 of the hour; *d*, mean wind speed for minutes 0–3 of the hour; *e*, atmospheric particulate lead. (Data other than lead from E. K. Bigg.)

The latter (mean monthly composite high volume samples) are higher in many cases because they represent ambient air lead concentrations with probable anthropogenic contributions rather than background alone. Comparison with other data from carefully selected sampling periods at the South Pole station<sup>14</sup> (mean 0.63 ng per SCM, range <0.19–1.2 ng per SCM) shows the Cape Grim figure to be higher. A possible explanation for the higher Cape Grim atmospheric lead concentrations may be the higher collection efficiencies achieved thereby.

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1. Chow, T. J., Earle, J. L. & Bennett, C. F. *Environ. Sci. Technol.* **3**, 737–740 (1969).
2. Duce, R. A. *Proc. Coll. Conservation Problems in Antarctica* 27–48 (1971).
3. Chester, R. & Stoner, J. H. *Nature* **245**, 27–28 (1973).
4. Chester, R. & Stoner, J. H. *Mar. Chem.* **2**, 157–188 (1974).
5. Woodruff, R. & Lech, J. F. *Analyt. Chem.* **44**, 1323–1325 (1972).
6. Noller, B. N., Bloom, H. & Parker, C. R. *5th Int. Conf. Atomic Spectroscopy* paper A12 (1975).
7. Noller, B. N. & Bloom, H. *Atmos. Environ.* **9**, 505–511 (1975).
8. Matousek, J. P. & Brodie, K. G. *Analyt. Chem.* **45**, 1606–1609 (1973).
9. Noller, B. N. & Bloom, H. *Analyt. Chem.* **49**, 346–348 (1977).
10. Katz, M. *Measurement of Air Pollutants. Guide to the Selection of Methods* 25 (WHO, Geneva, 1969).
11. Seeley, J. L. & Skogerboe, R. K. *Analyt. Chem.* **46**, 415–421 (1974).
12. Willeke, K. & Whitby, K. T. *Air Pollut. Contr. Ass. J.* **25**, 529–534 (1975).
13. Feely, H. W., Toonkel, L. E. & Schonberg, M. *U.S. Earth Sci. Appendix*, UC 11 B-1-B-149 (1976).
14. Zoller, W. H., Gladney, E. S. & Duce, R. A. *Science* **183**, 198–200 (1974).

## Palaeoanisotropy in the upper mantle

CARTER<sup>1</sup> has reviewed advances in understanding the steady-state flow of rocks with particular reference to the behaviour and anisotropic alignment of rocks in the upper mantle. Some form of high-temperature creep is clearly responsible for the distribution of continents and their first-order structures<sup>2</sup>. The difficulty is specifying the exact mechanism and the possible anisotropic alignments. Appropriate upper mantle temperatures and pressures can be achieved in the laboratory<sup>3</sup>, but any results must be extrapolated to rates of deformation lower by some five or six orders of magnitude. Carter *et al.*<sup>2</sup> conclude, although there are conflicting views<sup>4</sup>, that the syntectonic recrystallisation suggested by Avé Lallemant and Carter<sup>5</sup> is the dominant deformational process. This will be most effective at the higher temperatures at the base of the lithosphere where crystals, orientated by the deformations, have their alignments fixed as the lithosphere cools. All the deformational processes suggested for the upper mantle are capable of orientating crystalline structures, and aligned azimuthal-anisotropy may be expected at any depth in the lithosphere.

It is difficult to make accurate observations of the upper mantle, but whenever the lithosphere has been examined in sufficient resolution it is found to be anisotropic. Occasionally upper mantle material can be examined in the laboratory. Peselnick *et al.*<sup>6</sup> measure up to 7% velocity anisotropy in lherzolite xenoliths from the Sierra Nevada, which are estimated on compositional grounds to have originated at 50 km depth in the upper mantle. The only way *in situ* information can be obtained, however, is from seismic observations. Existing observations of azimuthal anisotropy in the upper mantle are of three types<sup>7</sup> (1)  $P_n$  velocity anisotropy, widely observed beneath the oceanic crust, and now beneath the Rhinegraben<sup>8</sup>, could be the result of as little as 5 km of anisotropic material beneath the mocho. (2) Velocity anisotropy of fundamental-mode surface-waves in the NAZCA Plate<sup>9</sup> is attributed to anisotropy extending down possibly to the top of the low-velocity-zone at about 125 km depth. (3) Polarisation anomalies of second-mode surface-waves across Eurasia<sup>10</sup> indicate at least 10 km of anisotropic material. These seismic observations establish anisotropy in the top few kilometres of the lithosphere, but, except for type (2) observed in one area only, give no indication of the vertical range.

There seem to be two main possibilities for the configuration of anisotropic alignments throughout the lithosphere in any given region. In uniform alignment, there will be little variation with depth of the alignment in the lithosphere, regardless of the tectonic history, if there is a deformation process which can modify the alignment, perhaps very slowly, to conform with the existing stress pattern over the past few million years.

In layered alignment, the alignment of the lithosphere will vary with depth if the alignment at the lower higher-temperature lithospheric boundary is permanently fixed as the lithosphere cools. This will result in the lithosphere having layers with different alignments corresponding to stress or flow patterns at some epoch in the past when the physical conditions were suitable for realignment to occur.

If syntectonic recrystallisation<sup>1,2,5</sup> is the dominant alignment process and there is no subsequent deformation, the layered configuration will persist, and palaeoanisotropy in the upper mantle will be preserved over long periods of time. In regions of comparatively young lithosphere such as that beneath the oceans, where the forces acting on the lithosphere have been substantially the same throughout its history, the alignment will be uniform. Carter<sup>1</sup> finds that the plastic deformation processes of Hess<sup>11</sup> and Francis<sup>12</sup> give rise to similar fabrics as syntectonic recrystallisation, and remarks that the question of which process is dominant is now less important. But, the process which produces the existing alignments and its period of

activity, is still fundamental to the interpretation of the anisotropic configuration of the lithosphere.

We see that to achieve a substantial realignment by any of the various processes in the given physical conditions, the relative time-constant determines which configuration exists. It is difficult to believe that any *a priori* arguments derived from surface experiments will clearly distinguish between configurations resulting from slow deformations in the upper mantle acting over hundreds of millions of years. Observations of the presence, absence, and variation of anisotropic alignments at different depths beneath oceans and continents may, therefore, be crucial in deciding the deformation processes and the tectonic history of the lithospheric upper mantle.

Numerical calculations<sup>10,13,14</sup> demonstrate that most seismological measurements are very little affected by even substantial amounts of anisotropy. The only diagnostic difference, between propagation through anisotropic and propagation through isotropic structures, is the coupling in the presence of anisotropy between polarisations in the radial-vertical plane and the transverse-horizontal direction. This coupling provides a technique for investigating deep anisotropy: body waves passing through anisotropic structures generate polarisation anomalies at each interface, which may be interpreted in terms of depth, thickness, orientation, and degree of anisotropy<sup>13,14</sup>. These anomalies are likely to be subtle and difficult to observe but they provide probably the only means of examining the *in situ* palaeoanisotropy in the lower lithosphere.

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1. Carter, N. L. *Rev. geophys. Space Phys.* **14**, 301–360 (1976).
2. Carter, N. L., Baker, D. W. & George, R. P. *Flow and fracture of rocks Geophys. Mono. Ser.* **16**, 167–190 (1972).
3. Peselnick, L. & Stewart, R. M. *J. geophys. Res.* **80**, 3765–3768 (1975).
4. Peselnick, L., Nicolas, A. & Stevenson, P. R. *J. geophys. Res.* **79**, 1175–1182 (1974).
5. Avé Lallemant, H. G. & Carter, N. L. *Am. J. Sci.* **270**, 218–235 (1971).
6. Peselnick, L., Lockwood, J. P. & Stewart, R. *J. geophys. Res.* **82**, 2005–2010 (1977).
7. Bamford, D. & Crampin, S. *Geophys. J. R. astr. Soc.* **49**, 1–8 (1977).
8. Bamford, D. *Geophys. J. R. astr. Soc.* **49**, 29–48 (1977).
9. Forsyth, D. W. *Geophys. J. R. astr. Soc.* **43**, 103–162 (1975).
10. Crampin, S. & King, D. W. *Geophys. J. R. astr. Soc.* **49**, 59–85 (1977).
11. Hess, H. H. *Nature* **203**, 629–631 (1964).
12. Francis, T. J. G. *Nature* **221**, 161–165 (1969).
13. Crampin, S. *Geophys. J. R. astr. Soc.* **49**, 9–27 (1977).
14. Keith, C. M. & Crampin, S. *Geophys. J. R. astr. Soc.* **49**, 225–243 (1977).

## Giant haloes in mica

MUCH attention has been given to the origin of 'giant' pleochroic haloes found in a number of minerals, especially micas<sup>1–6</sup>. Normal pleochroic haloes are due to the effects of radiation damage from  $\alpha$ -particles emitted from small inclusions at the centre of each halo, and the largest radius of the halo is determined by the range of the  $\alpha$ -particle of highest energy, which is that from <sup>212</sup>Po in the case of a thorium-rich inclusion. These have an initial energy of 8.78 MeV. The giant haloes seem to be of two kinds. In Gentry's sample<sup>7</sup> ~40% have a discrete radius ~54  $\mu$ m and he reports them to be invariably associated with dense thorium haloes. This group appears properly to be associated with production by the well-known but rare (~1.3  $\times 10^{-4}$ ) pair of long range  $\alpha$ -particle groups from <sup>212</sup>Po at ~10.5 MeV. The remaining giant haloes have a monotonic distribution in radius extending up to 110  $\mu$ m; they have been interpreted in terms of diverse processes such as extinct radiations of higher energy, proton knock-ons by  $\alpha$ -particles,

knock-ons from neutrons, channelling, migration of the parent radioactive material, but we report one natural and simple explanation which seems not to have been considered.

The giant haloes (illustrated, for example, in ref. 4) are also outstanding in that there is a large inclusion. Such large inclusions in mica, which is well known for the readiness with which it may be cleaved, seem very likely to be surrounded by a cleavage crack lying in the natural crystallographic cleavage plane, and presumably filled principally with H<sub>2</sub>O. The plane of the crack would pass close to the inclusion centre. Such a crack could well be initiated by changes in temperature or pressure at some stage during the life of the specimen, or possibly by swelling of the inclusion due to the high degree of radiation damage that it receives. If cracks radiating from the inclusion have propagated in planes approximately normal to the natural cleavage plane, as apparently happens in the haloes illustrated in ref. 3 and in Fig. 2 of ref. 4, then cracking parallel to the natural cleavage is extremely likely.

It seems reasonable that the surrounding crack in the cleavage plane could have an opening height,  $h \geq 0.1 \mu$ m at the boundary of a large inclusion, and extend up to perhaps 50  $\mu$ m outwards in the case of the largest inclusions. (Cracks with smaller openings, could possibly heal gradually, if a little plastic deformation of the mica can occur, and they are not kept open by intrusion of solid particles. With larger openings, stabilisation of the opening by inflow of debris becomes more likely. The clearly visible cracks normal to the cleavage plane (shown in refs 3, 4) must have  $h$  well exceeding our suggested lower limit.)

Cracks such as we consider here would help extend the volume exposed to radiation damage in two ways: (1) by allowing  $\alpha$ -particles entering or crossing the crack nearly parallel to the cleavage plane to travel a significant fraction of their range in H<sub>2</sub>O; and (2) by enhancing the capabilities of diffusion of the radon isotopes in radioactive equilibrium with the inclusion, some of which will be liberated following the recoil at their formation from their parent radium nucleus.

To be effective in extending the range of the  $\alpha$ -particles the crack has to possess sufficient height so that  $\alpha$ -particle scattering in the water can be ignored. For this simplifying approximation to be adequate it is necessary that  $h \geq 5 \times 10^{-4} x^{3/2}$  where  $x$  is the path length in H<sub>2</sub>O and both  $h$  and  $x$  are measured in  $\mu$ m. The range extension thus endowed to the  $\alpha$ -particles is close to 50% of the distance travelled in H<sub>2</sub>O since  $\alpha$ -particle ranges in H<sub>2</sub>O are close to twice those in both biotite and muscovite micas. The proportion of  $\alpha$ -particle tracks stopping in the crack plane that have a range extension appropriate to maximum passage through H<sub>2</sub>O will be of the order of  $h/2a$ , where  $a$  is the radius of the inclusion; this figure can, of course, be much larger than  $1.3 \times 10^{-4}$  which is the abundance of the naturally occurring 10.5 MeV long-range  $\alpha$ -particles.

With a half-life of ~52s, <sup>220</sup>Rn liberated from a thorium-rich inclusion has sufficient time to diffuse over the full extension of the volume of the crack, taking a diffusion coefficient equal to ~10<sup>-5</sup> cm<sup>2</sup>s<sup>-1</sup>, so that the polonium  $\alpha$ -particles, in particular, should have an origin that is more extended than the volume of the inclusion. This is also true for the uranium-rich inclusions, where <sup>222</sup>Rn has a half-life of 3.8 d. Such diffusion is very obvious in 'thorium stars' produced by radioactive impurities in nuclear emulsion. These are tracks of successive  $\alpha$ -particles produced in the decay chain from single nuclei of <sup>224</sup>Ra and <sup>228</sup>Ra which have an abundance of ~3  $\times 10^4$  cm<sup>-3</sup> in normal nuclear emulsion, Brown and Fowler, in ref. 8.

With each of these mechanisms the halo produced should not be spherical. It should have a radius of normal magnitude perpendicular to the cleavage plane. In the case of enhancement by  $\alpha$ -particle propagation along the plane of the crack, the fully enhanced radius of the halo should be restricted to a band of thickness  $\Delta Z \sim h$  the crack opening, whereas if enlarged by radon diffusion the enhancement would spread over a thickness  $\Delta Z$  of the order of the range of the subsequent polonium  $\alpha$ -particles. Quantitative information about the form of the



halo in this third dimension would be most valuable in attempting to determine which mechanism is in fact important in the generation of these giant haeles.

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1. Hodgson, P. E. *Nature* 267, 581–582 (1977).
2. von Wimmersperg, U. & Sellschop, J. P. F. *Phys. Rev. Lett.* 38, 886–888 (1977).
3. *GBL Physics Today* 29, 17–20 (1976).
4. Gentry, R. V. A. *Rev. nuc. Sci.* 23, 347–362 (1973).
5. Gentry, R. V. *et al. Phys. Rev. Lett.* 37, 11–15 (1976).
6. Sparks, C. J., Jr., Raman, S., Yakel, H. L., Gentry, R. V. & Krause, M. O. *Phys. Rev. Lett.* 38, 205–208 (1977).
7. Gentry, R. V. *Science* 169, 670–673 (1970).
8. Powell, C. F., Fowler, P. H. & Perkins, D. H. *The Study of Elementary Particles by the Photographic Method* 136–137 (Pergamon, London, 1959).

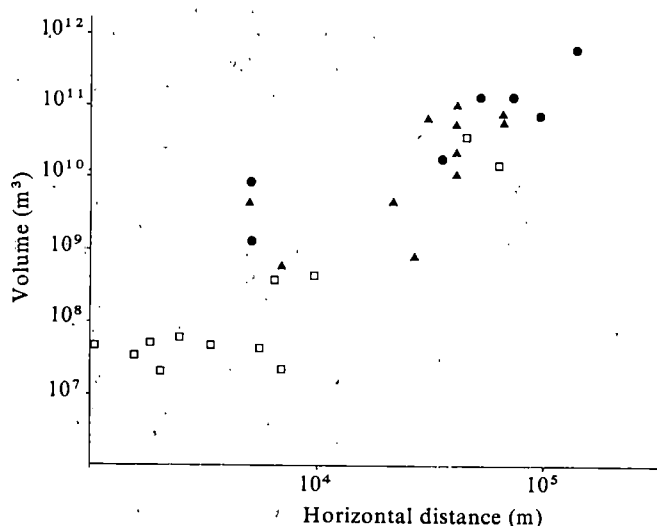
## Mobility of pyroclastic flows

THE mobility of pyroclastic flows has been described as spectacular<sup>1</sup>, in particular because of their ability to surmount obstacles in their path. It is relatively easy to estimate the minimum velocities required to climb a given height, if frictional losses are ignored. We have used data on well documented large cold rock avalanches to estimate the magnitude of frictional losses, and used these estimates to calculate velocities for some large pyroclastic flows. We argue that frictional losses in large pyroclastic flows must be comparable with, or less than, those in the most mobile rock avalanches, and calculate from data on heights climbed that velocities of large pyroclastic flows may be very high, up to 140 m s<sup>-1</sup>. We also conclude that the height of the eruption column giving rise to a large pyroclastic flow need only be a few hundred metres in some cases.

It is difficult to investigate the mobilities of large pyroclastic flows, such as those forming ignimbrites, since none has ever been observed in eruption. Only a few relatively small *nuée ardente* eruptions have been observed directly. Some of the largest ignimbrites have volumes of the order of 100 km<sup>3</sup>, extend for horizontal distances over 100 km (ref. 2), have climbed obstacles up to 600 m in height and have been estimated to travel at speeds up to 100 m s<sup>-1</sup> (ref. 3).

The scanty previous literature on mobility of pyroclastic flows has been summarised by Sparks<sup>3</sup> and Miller and Smith<sup>1</sup>.

Fig. 1 Horizontal distance travelled for well documented large rock avalanches and ignimbrites plotted against volume. □, rock avalanches; ▲, Central Andes ignimbrites; ●, other ignimbrites.



We have obtained some further data from the Central Andes, where three ignimbrites are known which have climbed obstacles up to 350 m high<sup>2</sup>. The way in which pyroclastic flows surmount obstacles has been disputed<sup>3</sup>. Recent observations on the upward concentrations of pumice clasts in ignimbrites imply high flow densities<sup>3</sup>; that is, pyroclastic flows may behave in a similar way to large rock avalanches.

For rock avalanches greater than about 10<sup>-3</sup> km<sup>3</sup>, Hsü showed that mobility is related to the volume—the largest being the most mobile<sup>4</sup>. Hsü explained the unusual mobility of large rock avalanches in terms of fluidisation of fine debris by entrapped air; Shreve in contrast suggested that rock avalanches travel on a cushion of trapped, compressed air<sup>5</sup>. Whatever the mechanism, the efficiency of the process seems to increase with the size of the moving body.

Many ignimbrites have volumes comparable with or greater than the largest known rock avalanches, some having volumes in excess of 100 km<sup>3</sup> (ref. 6). The very high mobility of the pyroclastic flows giving rise to such deposits can be inferred from Fig. 1, where horizontal distance travelled is plotted against volume for pyroclastic flows and large rock avalanches. The large volume ignimbrites plot in a distinctly more mobile field than all but the largest of the rock avalanches.

Although there are a few direct measurements on the velocities of small *nuée ardentes*—up to 60 m s<sup>-1</sup> (refs 7 and 8)—the velocities of the much larger volume pyroclastic flows can only be inferred from their climbing ability. Simple calculations, based on the conversion from potential to kinetic energy, can be done which relate the horizontal velocity to the height climbed. By this means, Sparks showed that a minimum velocity of the order of 100 m s<sup>-1</sup> is required for a pyroclastic flow to climb a 600 m high obstacle<sup>3</sup>. Such calculations take no account of frictional losses. We have obtained crude estimates of these losses in pyroclastic flows by looking at data for much better known rock avalanches. In the case of a body of rock travelling down one slope and up an opposing one, an estimate of the overall percentage frictional loss ( $F$ ) can be derived, from the interconversion of potential energy and kinetic energy, by including frictional loss in the appropriate formulae

$$\begin{array}{lcl} \text{kinetic energy gained} & = & \text{potential energy lost} \\ \frac{1}{2}mv^2 & & \left(\frac{100-F}{100}\right)mgh_1 \end{array}$$

$$\begin{array}{lcl} \text{potential energy gained} & = & \text{kinetic energy lost} \\ mgh_2 & & \left(\frac{100-F}{100}\right)\frac{1}{2}mv^2 \end{array}$$

where  $m$  = mass;  $v$  = velocity,  $F$  = percentage frictional loss,  $h_1$  = height lost;  $h_2$  = height gained

$$\text{from which} \quad F = 100 \left(1 - \left(\frac{h_2}{h_1}\right)^{\frac{1}{2}}\right)\%$$

Clearly, the calculated value of  $F$  will be increased if the flow travels a large horizontal distance between the two slopes, or if the main direction of travel is oblique to the slope climbed. Relevant data for the best known rock avalanches are listed in Table 1. In the more mobile of these  $F$  is between 40 and 60%. In Fig. 2 we have plotted the theoretical relationship between horizontal velocity and height climbed for values of  $F$  between 0 and 60% (solid lines).

Taking the data summarised in Fig. 1 into account, large pyroclastic flows are likely to have values of  $F$  comparable to, or even lower than, the most mobile rock avalanches, that is, in the range 30–40%. On this basis, the velocities required for the obstacle climbing ignimbrites plotted on Fig. 2 would be in the range 85 to 140 m s<sup>-1</sup>. These values are realistic: the errors not

Table 1 Height data and values of  $F$  for documented large rock avalanches

Name	Volume (m <sup>3</sup> )	Height descended $h_1$ (m)	Height climbed $h_2$ (m)	$F$ (%)	Remarks
Elm <sup>4</sup>	$1.1 \times 10^7$	450–560	100	50–55	
Little Tahoma Peak <sup>9</sup>	$1.1 \times 10^7$	1,250–1,890	40–90	75–85	Climbed oblique slope
Sherman <sup>10</sup>	$2.8 \times 10^7$	600	140	50	
Madison <sup>11</sup>	$2.9 \times 10^7$	400	120	45	
Frank <sup>5</sup>	$3.7 \times 10^7$	760–920	120	60–65	
Silver Reef <sup>6</sup>	$2.2 \times 10^8$	1,000	45	80	Travelled a long horizontal distance
Blackhawk <sup>6</sup>	$2.8 \times 10^8$	1,220	60	80	Travelled a long horizontal distance
Saidmarreh <sup>11</sup>	$4.3 \times 10^9$	1,520	460–610	35–45	

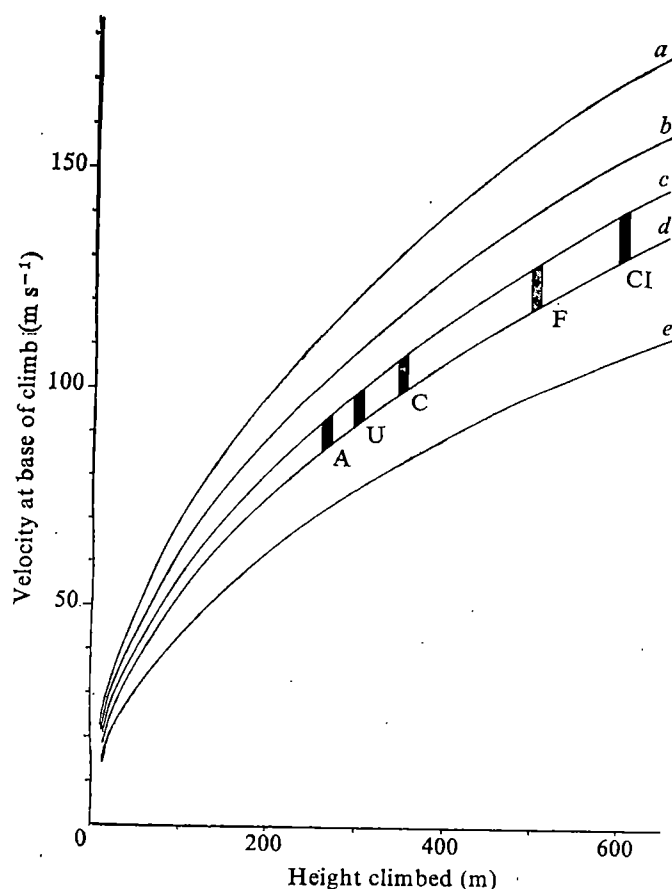
taken into account in our simple model would only increase the values of  $F$ , and therefore the velocities required. Such high velocities have two important implications.

First, the emplacement of even a very large ignimbrite sheet could take place in a very short time, that is minutes rather than hours. This is particularly relevant in considering the mechanisms of transport of pyroclastic flows, and the origin of textures within them, such as the sorting of pumice fragments. Second, if values for  $F$  for pyroclastic flows giving rise to ignimbrites were around 30–40%, indicating efficient lubrication of the flow by fluidised fine grained material, it is not necessary to invoke collapse from eruption columns of exceptional height

Fig. 2 Theoretical relationship between horizontal velocity at base of climb and height climbed for values of  $F$ : a,  $F = 60\%$ ; b,  $F = 50\%$ ; c,  $F = 40\%$ ; d,  $F = 30\%$ ; e,  $F = 0\%$ ; using the

expression  $V = 10 \left( \frac{2gh_2}{100F} \right)^{1/2}$ . Also plotted are estimated velocity

ranges for individual ignimbrites assuming values of  $F$  between 30 and 40%: A, Aniakchak<sup>1</sup>; U, Ujina<sup>2</sup>; C, Carcote<sup>2</sup>; F, Fisher<sup>1</sup>; CI, Campanian<sup>3</sup> and Ito<sup>3</sup>.



to account for the high speeds. For example, collapse from a comparatively modest column 400–500 m high would be sufficient to account for the mobility of the Ujina ignimbrite. In other cases, where the pyroclastic flow travelled a substantial distance horizontally before surmounting an obstacle, the frictional losses would have been greater, and therefore the mobility of the flow must have been achieved by collapse from an eruption column of comparatively great height.

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1. Miller, T. P. & Smith, R. L. *Geology* 5, 173–176 (1977).
2. Baker, M. C. W. thesis, Open Univ. (1977).
3. Sparks, R. S. J. *Sedimentology* 23, 147–188 (1976).
4. Hsü, K. J. *Bull. geol. Soc. Am.* 86, 129–140 (1975).
5. Shreve, R. L. *Spec. Pap. geol. Soc. Amer.* 108, (1968).
6. Guest, J. E. *Bull. geol. Soc. Am.* 80, 337–362 (1969).
7. Moore, J. G. & Melson, W. G. *Bull. Volcanol.* 33, 600–620 (1969).
8. Hobbs, P. V., Radke, L. F. & Stith, J. L. *Science* 195, 871–873 (1977).
9. Crandell, D. R. & Fahnstock, R. K. *Bull. U.S. geol. Serv.* 1221-A (1965).
10. Shreve, R. L. *Science* 154, 1639–1643 (1966).
11. Kent, P. E. J. *Geol.* 74, 79–83 (1966).

## Ratio of prey to predators in community food webs

WHETHER the diversity of resources limits the diversity of consumers, and specifically, whether the number of kinds of prey limits the number of kinds of predators, has been of continuing interest in theoretical ecology and wildlife management<sup>1–3</sup>. Food webs from the ecological literature were collected in machine readable form to study this question empirically. We report here that in community food webs, the ratio of the number of kinds of prey to the number of kinds of predators seems to be constant, near 3/4. This invariance has not been noticed in earlier studies of individual cases.

Before analysis, food webs were characterised as one of three types—community, sink and source. Community food webs describe all kinds of organisms (possibly restricted to some location, size or taxa) in a habitat, without reference to the eating relations among them. Sink food webs describe all the prey taken by a set of one or more selected predators, plus all the prey taken by the prey of those predators, and so on. Source food webs describe all the predators on a set of one or more selected prey organisms, plus all the predators on those predators, and so on. Sink and source food webs, hypothetical or schematic constructions, and avowedly incomplete, partial or tentative food webs were excluded from further study. Fourteen community food webs were thus selected. The complete data and individual cases will be discussed elsewhere<sup>4</sup>. When the report of a food web contained ambiguous or uncertain information about a

feeding relation, the web was included in two versions, one based only on the unambiguous information and the other incorporating the additional uncertain or probable eating relations. The analysis presented here based on all versions, makes no claim that the data points are statistically independent and attaches no probability values to the statistics calculated.

The food webs describe the diets or predators not of individual organisms but of kinds of organisms. A 'kind of organism' may be a stage in the life cycle or a size class within a single species, or a collection of functionally or taxonomically related species, according to the practice of the original report. The numbers in the following analyses refer to these ecologically defined kinds of organisms, not necessarily to any conventional taxonomic unit. A predator is defined as a kind of organism that consumes at least one kind of organism included in the food web. A prey is defined as a kind of organism that is consumed by at least one kind of organism in the food web. Some kinds of organisms may be both predators and prey.

In community food webs, the number  $m$  of prey is very nearly proportional to the number  $n$  of predators (Fig. 1). A least squares regression of  $m$  against  $n$  gives

$$m = 1.79 + 0.71n \quad (1)$$

The sample standard deviation of the regression coefficient is 0.07 and the linear correlation coefficient between  $m$  and  $n$  is 0.90. The standard error of estimate, or sample standard deviation from regression, is 4.62. As is obvious from Fig. 1,

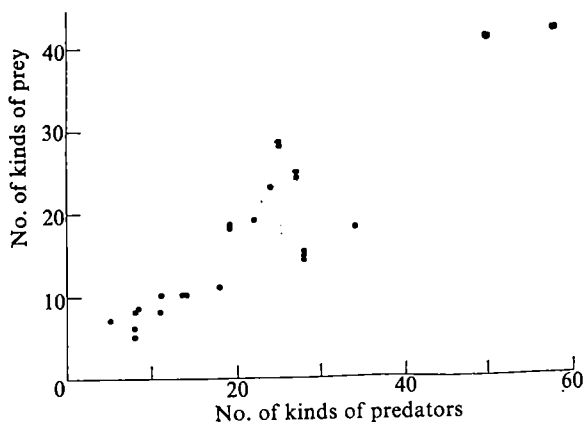


Fig. 1 The number of kinds of prey and the number of kinds of predators in community food web versions.

the regression may be well approximated by a straight line through the origin. The least squares regression is

$$m = 0.77n \quad (2)$$

The proportionality between the number of prey and the number of predators in Fig. 1 is based on 24 versions of 14 food webs reported over a period of decades. When the food webs were collected and encoded it was not known that such a simplicity would emerge. It therefore seems likely that this invariance in the proportions of predators and prey represents a fact about nature, rather than an artefact of collusion or convention.

Given that the proportion of prey to predators is a scale-invariant feature of community food webs, the proportion can be predicted quantitatively from other facts. For a given food web with  $m$  prey and  $n$  predators, let  $A$  be the number of predator-prey couples. (If  $X$  eats  $Y$  and  $Y$  eats  $X$ , the couples  $(X,Y)$  and  $(Y,X)$  are counted as distinct. If  $X$  eats  $X$ ,  $(X,X)$  also counts as a couple. In the conventional graphical representation of a food web,  $A$  is the number of directed arrows from prey to predator.) Then within any food web

$$A = (\text{average prey per predator}) \times n \\ = (\text{average predators per prey}) \times m \quad (3)$$

The grand mean over all 24 community food web versions, weighting each food web equally, of the average prey per predator is 2.418; the grand mean of the average predators per prey is 3.199. If these means apply to each food web, then substitution into equation (3) predicts

$$m/n = 2.418/3.199 = 0.756 \quad (4)$$

which differs trivially from the least squares regression in equation (2).

The simplicity of the argument from the proportionality between  $m$  and  $n$  to equation (4) may raise a suspicion that its success depends on an arithmetical fact rather than on the observed invariance of proportions of predators and prey in nature. A numerical example disproves this suspicion. Suppose a sample of community food webs consisted of two food webs. Suppose the first food web matrix had  $m_1=8$  prey,  $n_1=6$  predators, and  $A_1=19.2$  predator-prey couples (neglecting the requirement that  $A_1$  be integer for the sake of argument). Then its (average predators per prey)<sub>1</sub> is 2.4 and its (average prey per predator)<sub>1</sub> is 3.2. Suppose the second food web matrix had  $m_2=4$ ,  $n_2=10$ , and  $A_2=16$ . Then its (average predators per prey)<sub>2</sub>=4.0 and (average prey per predator)<sub>2</sub>=1.6. Then the grand mean over both food webs of the average predators per prey is 3.2 and the grand mean of the average prey per predator is 2.4, which are close enough to the observed. But the straight line through the pairs  $(n, m)$  satisfies  $m=14-n$ . Only because nature assures a constant proportion of prey to predators do the grand mean of the average predators per prey and the grand mean of the average prey per predator apply to all food webs.

If the ratio of prey to predators in community food webs is a constant of the order of 3/4, then dividing equation (3) by  $n$  leads to the prediction that a regression (Fig. 2) of average prey per predator against average predators per prey should be a straight line through the origin with slope 3/4. The regression coefficient of a straight line through the origin is 0.69, not far from 3/4.

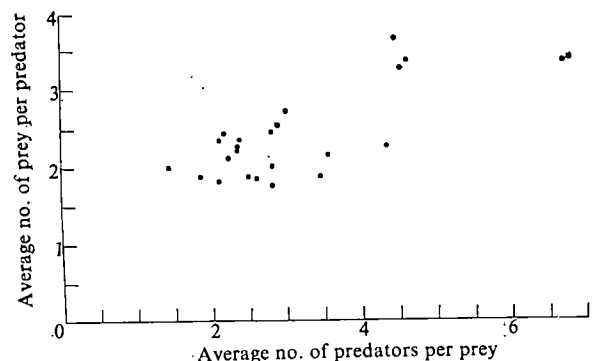


Fig. 2 The average number of kinds of prey per kind of predator and the average number of kinds of predators per kind of prey in community food web versions.

In conclusion, in community food webs, the number of kinds of prey, as operationally defined by field ecologists, approximates 3/4 the number of kinds of predators. This results from the study only of an ensemble of food webs, rather than of individual cases.

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1. Haigh, J. & Maynard Smith, J. *Theor. Pop. Biol.* 3, 290–299 (1972).
2. Levin, S. A. & Paine, R. T. *Proc. natn. Acad. Sci. U.S.A.* 71, 2744–2747 (1974).
3. Sullivan, A. L. & Shaffer, M. L. *Science* 189, 13–17 (1975).
4. Cohen, J. E. *Food Webs and Niche Space*, (Princeton University Press, Princeton, in the press).

## Inhibition of drinking by intrahypothalamic administration of morphine

MORPHINE inhibits the release of acetylcholine (ACh) within both the peripheral<sup>1–4</sup> and central<sup>5–9</sup> nervous systems. This would suggest that a major effect of acute morphine treatment should be inhibition of behaviour mediated by central cholinergic transmission, but few behavioural demonstrations of this antagonism exist. We report here that intrahypothalamic injections of equimolar concentrations of morphine effectively antagonise the muscarinic-cholinergic drinking response<sup>10–13</sup> elicited by injection of 4.0 nmol of carbachol into the hypothalamus. In addition, intracranial (i.c.) injections of morphine also blocked drinking elicited by several other dipsogenic stimuli, while not affecting eating. The results suggest that morphine not only inhibits the release of ACh but also may block the postsynaptic muscarinic receptor. This inhibitory effect of i.c. morphine may, furthermore, provide a simple behavioural screening test for central opiate activity.

The effects of intrahypothalamic applications of morphine on water consumption were investigated in two experiments involving 44 adult male Sprague-Dawley rats. In each experiment, stainless steel cannulae (24 gauge) were stereotactically implanted into the perifornical hypothalamus<sup>14</sup> (PFH) of anaesthetised (Nembutal, 40 mg per kg body weight intraperitoneally) rats. Each subject was allowed at least one week to recover from surgery before testing was initiated. Drugs were administered i.c. as free bases in 1- $\mu$ l volumes through a 31-gauge hypodermic needle directly connected to a 50- $\mu$ l syringe. A repeating dispenser (Hamilton, PB600–1) was used to ensure reliability of injection volumes. Before any tests of morphine, stable baseline drinking to the dipsogenic stimuli was ensured across several days of testing. Ground rat chow and water intake were measured after 1-h periods of both control and experimental treatment conditions. Tests of drug antagonism were conducted by administering an equimolar dose of the antagonist to the same brain area 5 min before the injection of the agonist.

In the first experiment, 28 rats were randomly divided into three groups. Eating and drinking were assessed in each group after the control injection of saline and the subsequent application of carbachol (Fig. 1). As previously reported<sup>12</sup>, carbachol elicited significantly more eating ( $F(1,25) = 15.35$ ,  $P < 0.01$ ) and drinking ( $F(1,25) = 67.72$ ,  $P < 0.01$ ) than the control manipulation.

On the following day, the antagonistic effect of morphine on carbachol-elicited drinking was investigated. The reversibility of morphine antagonism of drinking was also tested in another group by the i.c. injection of the narcotic antagonist, naloxone, 5 min before the morphine treatment. Although there were no significant differences in food intake between the three groups (Table 1), there was a significant overall effect on water consumption ( $F(2,25) = 13.17$ ,  $P < 0.01$ ). Post hoc *t* tests indicated no difference in drinking between the saline pretreated carbachol (SSC) and naloxone-morphine pretreated carbachol (NMC) groups, while each of these groups differed from the saline-morphine pretreated carbachol (SMC) group ( $P < 0.01$ ). Thus, i.c. morphine blocked carbachol-elicited drinking, while not significantly affecting eating. Furthermore, this antagonism of drinking was reversed by pretreatment with naloxone.

Since cholinergic influences have also been implicated in the mediation of deprivation-induced drinking<sup>13</sup>, 18 of the above rats were deprived of food and water for 24 h and tested for

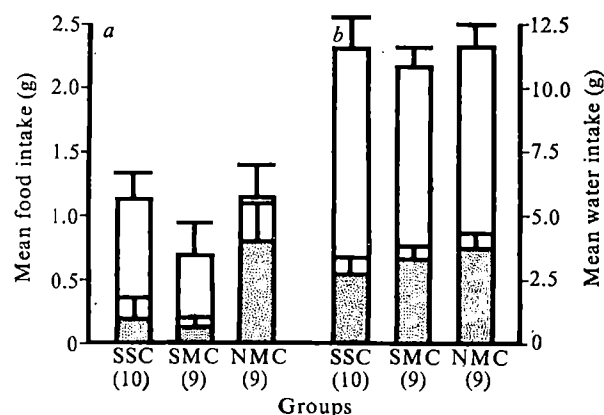


Fig. 1 Mean food (a) and water (b) intake ( $\pm$  s.e.m.) by rats during the 1-h following the injection of carbachol (4.0 nmol) and the preceding control injection of 1  $\mu$ l of normal saline (stippled area). This test was conducted after the rats had received three previous daily injections of carbachol and saline and represents the animals' response during the fourth trial. Group designations refer to the morphine test, which was conducted on the following day: S, saline; C, carbachol; N, naloxone; M, morphine. Numbers in parentheses are number of animals tested.

morphine antagonism of drinking and eating. That i.c. morphine effectively antagonised drinking while not significantly affecting eating (Table 2) indicates that this adipsic effect is not due to nonspecific depression of behaviour.

In the second experiment, 16 experimentally-naïve rats were randomly assigned to two groups to assess the effect of i.c. morphine across several other paradigms of drinking. Since morphine has been reported to inhibit the uptake of carbachol into brain slices<sup>15</sup>, carbachol was administered before morphine in the first test. Although carbachol was injected well before morphine and undoubtedly had been taken up by central neurones (as shown by a normal drinking latency of 5 min), drinking was still significantly reduced by the opiate (Table 3). The second test examined the effects of i.c. morphine treatment on drinking elicited by hypertonic saline<sup>13</sup>. Again i.c. morphine effectively obviated the drinking response to this dipsogenic challenge (Table 3). Pretreatment with equimolar doses of morphine also antagonised drinking elicited by the potent dipsogenic peptide, angiotensin II<sup>16</sup> (Table 3).

To provide additional control for nonspecific effects of morphine on consummatory behaviour, two additional tests were conducted. Since morphine seemed to depress eating somewhat in previous experiments (Tables 1 and 2), the effects of

Table 1 Effects of intrahypothalamic morphine and naloxone pretreatments on carbachol-elicited eating and drinking

Group	N	Food intake (g per h)	P	Water intake (g per h)	P
SSC	10	1.1 $\pm$ 0.3	NS	12.0 $\pm$ 1.1	0.01
SMC	9	0.5 $\pm$ 0.3		2.7 $\pm$ 1.5	
NMC	9	0.6 $\pm$ 0.2		9.1 $\pm$ 1.7	

Mean food and water intake in grams ( $\pm$  s.e.m.) by rats during 1 h following the injection of carbachol (4.0 nmol). Group SSC received injections (1  $\mu$ l) of normal saline 10 and 5 min before carbachol, while groups SMC and NMC were injected with saline (1  $\mu$ l) followed by morphine (4.0 nmol) or naloxone (4.0 nmol) followed by morphine (4.0 nmol), respectively, 10 and 5 min before carbachol. Although there were no overall differences in food intake, group SMC drank significantly less water than group SSC or group NMC. There was no significant difference in water intake between groups SSC and NMC. Statistical inferences were made using analysis variance techniques with post hoc comparisons made by *t* tests. NS, not significant.



**Table 2** Effects of intrahypothalamic morphine treatment on eating and drinking elicited by 24 h food and water deprivation

Treatment	N	Food intake (g per h)	P	Water intake (g per h)	P
Saline	9	5.0±0.7	NS	6.2±0.8	0.01
Morphine	9	3.4±0.8		1.2±0.4	

Following the test of morphine inhibition of carbachol-elicited eating and drinking (Table 1), 18 rats were deprived of food and water for 24 h. The saline group received 1 µl of normal saline, while the morphine group was injected with 4.0 nmol of morphine 5 min before being presented with food and water. Intake in grams (±s.e.m.), was assessed during the 1-h period following the initial presentation of food and water. Although there was no difference between groups in food intake, morphine-treated animals drank significantly (*t* test) less water than did those given saline. NS, not significant.

i.c. morphine on eating and drinking elicited by 48-h food deprivation were investigated. It is well known that eating is reduced when drinking is severely inhibited. Therefore, the purpose of this test was to maintain hydration at near normal values with drinking being elicited by increased thirst subsequent to food consumption. As indicated in Table 3, there was no difference in food intake between the saline and morphine groups. Water intake, however, was still severely inhibited by the morphine treatment. To assess the effects of pretreatment with a non-addicting, non-analgesic stereoisomer, dextrophan was substituted for morphine in this final test. To control for the possibility of tolerance to the opiates, the groups were reversed with the dextrophan group never having been injected with opiates. In contrast to the previously-observed effects of morphine, i.c. dextrophan did not reduce either eating or drinking in these satiated rats (Table 3).

These experiments demonstrate a potent inhibitory effect of

**Table 3** Effects of intrahypothalamic opiates on eating and drinking elicited by various dipsogenic stimuli

Group	N	Food intake (g per h)	P	Water intake (g per h)	P
C-S	8	1.4±0.5	NS	10.1±0.2	0.01
C-M	8	1.1±0.7		2.2±0.8	
S-HS	8	0.1±0.1	NS	11.4±1.5	0.01
M-HS	8	0.4±0.3		2.4±1.2	
S-A	8	0.5±0.3	NS	19.8±2.2	0.01
M-A	8	0.6±0.3		7.2±1.8	
FD-S	8	6.1±0.8	NS	5.8±1.3	0.01
FD-M	8	7.4±0.8		0.9±0.2	
S-C	8	1.1±0.4	NS	9.5±2.3	NS
D-C	8	1.6±0.3		11.0±1.9	

Mean food and water intake was measured in grams (±s.e.m.) during 1-h periods following various dipsogenic stimuli. During the first test, intracranial (i.c.) injections of carbachol (C; 4.0 nmol) were administered 10 min before the i.c. injection (1 µl) of saline (S) or 4.0 nmol morphine (M) with the rats being presented with food and water 5 min after the last injection. Six days later, i.c. M (4.0 nmol) or S (1 µl) was administered 5 min before the injection (2 ml; subcutaneously) by hypertonic (10%) saline (HS). After a 3-d latency, the antagonistic effects of i.c. M (4.0 nmol) or S (1 µl) were assessed when administered 5 min before i.c. angiotensin II (A; 4.0 nmol). Six days later, the rats were food-deprived (FD) for 48 h and presented with food and water 5 min after the injection (i.c.) of M (4.0 nmol) or S (1 µl). The last test, conducted 6 d later, assessed the effects of i.c. dextrophan (D; 4.0 nmol) and S (1 µl) as pretreatments (5 min) on eating and drinking elicited by 4.0 nmol (i.c.) C. Statistical significance is based on *t* tests. NS, not significant.

centrally-applied morphine across several paradigms of drinking. This effect was not due to nonspecific behavioural disruption, since the rats were alert and ate amounts of food comparable to the control groups. Furthermore, the antagonism of drinking seemed to be due to the narcotic properties of morphine, since it was reversed by pretreatment with naloxone. This hypothesis is strengthened by the lack of effect observed when the stereoisomer, dextrophan, was substituted for morphine.

These inhibitory effects of morphine on drinking are similar to results previously reported following administration of atropine or scopolamine and may reflect antimuscarinic cholinergic activity. Although pretreatments with atropine have been reported to block drinking elicited by carbachol<sup>11</sup>, hypertonic saline<sup>13</sup>, water deprivation<sup>13</sup>, and schedule-induced polydipsia<sup>17</sup>, atropine is apparently not nearly as potent as morphine in paradigms that do not involve exogenous cholinergic dipsogens. Furthermore, atropine has been reported to be ineffective against drinking elicited by angiotensin II<sup>18</sup>, while pretreatment with morphine effectively blocked this behaviour. A possible difference between the central nervous system activity of morphine and atropine may be the effect of these drugs on the release of ACh. Thus morphine, which has been extensively reported to inhibit the release of ACh, may exert its antagonism of drinking through both the inhibition of release and postsynaptic muscarinic blockade. Thus, drinking in paradigms not involving exogenous cholinergic dipsogens, such as water deprivation, are not as readily antagonised by atropine. Conversely, morphine will readily inhibit the release of ACh and thus block the cholinergic components of deprivation-induced drinking. The reduction of drinking following i.c. angiotensin by morphine pretreatment may furthermore reflect a cholinergic component in the mediation of this behaviour<sup>18</sup>.

Antimuscarinic cholinergic effects of morphine have been suggested by several observations. The muscarinic cholinergic agonist, pilocarpine, stimulates transmission in the superior cervical ganglion of the cat<sup>19</sup>, and this effect, furthermore, is blocked by atropine as well as by morphine<sup>19</sup>. The observation that muscarinic receptor blockers reduce symptoms of precipitated morphine withdrawal<sup>20</sup> also suggests antimuscarinic activity of morphine is provided by the observation that tolerance to epileptiform electroencephalogram patterns following microinjections of morphine into the amygdala is accompanied by cross tolerance to scopolamine<sup>21</sup>.

We are extending these investigations to other opiate and opioid drugs and investigating the effect of systemically-administered morphine on these various dipsogenic challenges. It is possible that these tests may lead to a simple behavioural test of central narcotic activity.

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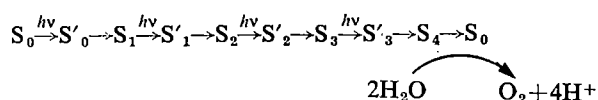
Received 9 June; accepted 19 September 1977.

1. Paton, W. D. M. *Br. J. Pharmac.* **12**, 119-128 (1957).
2. Schaumann, W. *Br. J. Pharmac.* **12**, 115-118 (1957).
3. Kosterlitz, H. W. & Wallis, D. I. *Br. J. Pharmac.* **26**, 334-344 (1966).
4. Pinsky, C. & Frederickson, R. C. A. *Nature, new Biol.* **231**, 94-96 (1971).
5. Beleslin, D. & Polak, L. J. *Physiol., Lond.* **177**, 411-419 (1965).
6. Jhamandas, K., Phillis, J. W. & Pinsky, C. *Br. J. Pharmac.* **43**, 53-66 (1971).
7. Labrecque, G. & Domino, E. F. *J. Pharmac. exp. Ther.* **191**, 189-200 (1974).
8. Clouet, D. H. & Williams, D. J. *J. Pharmac. exp. Ther.* **188**, 419-428 (1974).
9. Sharkawi, M. & Schulman, M. P. *J. Pharm. Pharmac.* **21**, 546-547 (1969).
10. Grossman, S. P. *Science* **132**, 301-302 (1960).
11. Levitt, R. A. *Psychom. Sci.* **15**, 274-276 (1969).
12. Chance, W. T. & Rosecrans, J. A. *Proc. Soc. Neurosci.* **2**, 286 (1976).
13. Block, M. L. & Fisher, A. E. *Physiol. Behav.* **5**, 525-527 (1970).
14. Pellegrino, L. J. & Cushman, A. J. *A Stereotaxic Atlas of the Rat Brain* (Appleton Century Crofts, New York, 1967).
15. Taylor, D. B., Creese, R. & Tzu-Chiau, L. J. *J. Pharmac. exp. Ther.* **165**, 310-319 (1969).
16. Epstein, A. N., Fitzsimons, J. T. & Simmons, B. J. *J. Physiol., Lond.* **200**, 98-100 (1969).
17. Burks, C. D. & Fisher, A. E. *Physiol. Behav.* **5**, 635-640 (1970).
18. Giardina, A. R. & Fisher, A. E. *Physiol. Behav.* **7**, 653-655 (1971).
19. Trendelenburg, U. *Br. J. Pharmac.* **9**, 481-487 (1954); **12**, 79-85 (1957).
20. Collier, H. O. J., Francis, D. L. & Schneider, C. *Nature*, **237**, 220-223 (1972).
21. Teitelbaum, H. *Nature* **267**, 452-453 (1977).

## EPR signals in chloroplasts responding to illumination sequence of four flashes

THE use of water as the electron donor and the consequent evolution of oxygen is one of the most important properties of photosynthesis by higher plants and algae. The properties of the enzyme systems involved in water oxidation are essentially unknown, however. Two new electron paramagnetic resonance (EPR) signals observed at low temperature in oxygen evolving chloroplasts are described here. The signals disappear and appear in response to short flash illumination, before freezing, of the chloroplasts, presumably due to changes in oxidation state of the components giving rise to the signals. The changes occur in response to a cycle of four flashes; it is suggested that they may reflect the oxidation states of the oxygen evolving system of the chloroplasts.

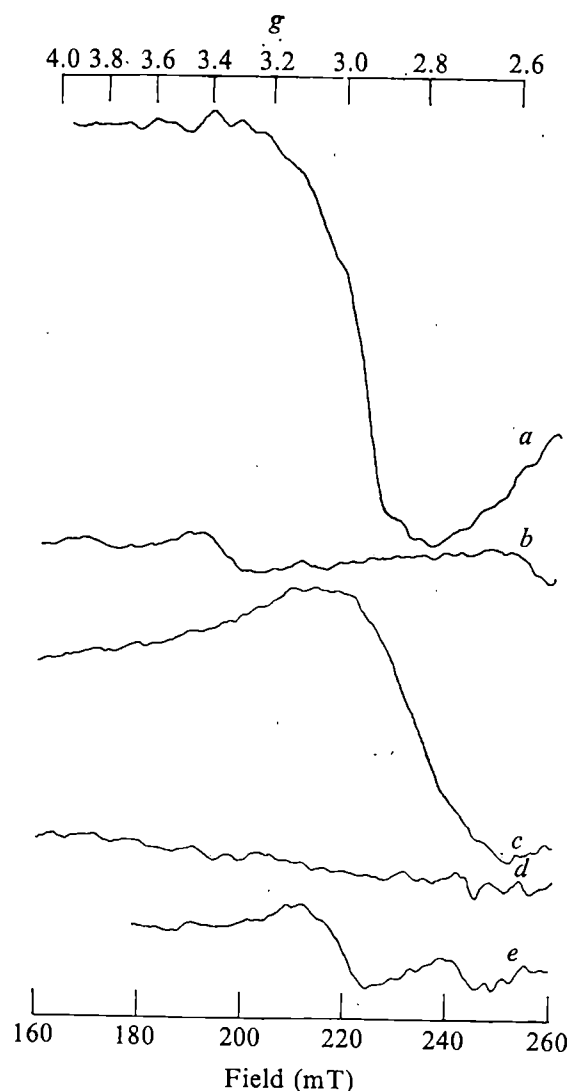
Manganese may be an important component of the oxygen evolving system<sup>1</sup>, but attempts to observe changes in the redox state of manganese associated with oxygen evolution have not been successful. Kinetic measurements<sup>2,3</sup> have shown that when chloroplasts are illuminated with short intense flashes of light, each of which causes only a single electron to be transported by each photosynthetic reaction centre, oxygen is evolved with a periodicity of four flashes. These experiments suggest that each water oxidising enzyme system accumulates four oxidising equivalents, one with each turnover of the photosystem II reaction centre, before reacting with water. This observation may be formalised in the 'S' state hypothesis of Kok *et al.*<sup>3</sup>



In kinetic experiments oxygen evolution normally shows a peak after the first three flashes, and then with a periodicity of four flashes, indicating that the distribution of the 'S' states is not random in the dark.  $S_1$  and  $S_0$  are thought to be most stable in the dark, with  $S_1$  predominating in most experiments<sup>4</sup>. The light induced progression between the 'S' states represents a change in redox state of the proposed enzyme, each step involving a single electron oxidation. As this enzyme may be expected to contain a transition metal ion complex it seems likely that these changes could be detected by EPR spectroscopy. Several studies of chloroplast electron transport using EPR and flash illumination have been reported but none has identified signals corresponding to the 'S' states<sup>5</sup>. Both optical and nuclear magnetic resonance (NMR) spectrometry have been used in attempts to identify the 'S' state components. Pulles *et al.*<sup>6</sup> and Mathis and Haveman<sup>7</sup> have reported optical changes at 310 nm responding to flash illumination with a periodicity of four. The chemical nature of the component involved is unknown. Wydrzynski *et al.*<sup>8,9</sup> observed changes in the relaxation rate of water protons showing response to flash illumination with a periodicity of four using NMR spectrometry: they presented evidence that their results are consistent with the participation of manganese in the flash-induced changes in the relaxation rate of water protons.

We have used flash illumination at room temperature followed by rapid freezing to 90 K in an attempt to trap the 'S' states, and low temperature (4.2–30 K) EPR spectrometry to detect new EPR signals showing a response to flash number, which would be expected from the oxygen evolving enzyme system, or a chloroplast component reflecting the redox state of this system.

Figure 1 shows the EPR spectra of a chloroplast preparation in the dark and after exposure to 0, 1, 2, 3, or 4, 20- $\mu$ s flashes from a 25-J xenon-arc lamp, the multiple flashes were spaced at 200-ms intervals. The samples were frozen within 1 s using an isopentane bath at 90 K and stored at 77 K until the spectra were recorded. The spectra show a signal in the dark at  $g = 3.09$ .



**Fig. 1** EPR spectra of broken spinach chloroplasts in the  $g = 3.0$  region. Broken chloroplasts were prepared in a Mn-free medium containing 1mM EDTA<sup>15,16</sup>. The chloroplasts which exhibited an uncoupled rate of oxygen evolution of 306  $\mu$ mol per mg chlorophyll per h were kept in the dark on ice for 0.5 h. The chlorophyll concentration was 1.55 mg ml<sup>-1</sup>. EPR spectra were recorded as described previously<sup>17,18</sup>. The conditions for each spectrum were as follows. Frequency 9.189 GHz, power 20 mW, modulation amplitude 1mT, scan rate 100 mT min<sup>-1</sup>, gain 500, temperature 10 K. The samples were illuminated with a, 0; b, 1; c, 2; d, 3 and e, 4 flashes. The spectra were recorded in the dark.

After one flash there is essentially no signal in the  $g = 3.0$  region. After a second flash a large signal appears centred at  $g = 2.79$ . The properties of this signal suggest that it arises from a different redox state to that giving rise to the dark signal. After the third flash there is again no signal while after the fourth flash, which in the 'S' scheme should regenerate the dark state, the original signal reappears at  $g = 3.07$ . This signal is, however, usually rather smaller than the initial dark signal.

These signals might be considered to correspond to the  $S_1$ ,  $S_2$ ,  $S_3$  and  $S_0$  states of Kok's model.  $S_4$  would not be seen as it reacts rapidly with water<sup>4</sup>. The rate of this reaction might, however, control the extent of regeneration of  $S_1$  by the fourth flash. Some decay of each stage will also occur between flashes, this and other problems well documented for the kinetic experiments will rapidly result in a loss of periodicity and the ability to detect the signals.

As described above, the response of these signals to flash illumination strongly suggests that they reflect the oxidation state of the water oxidising enzyme system. The EPR properties

of these signals are extremely unusual, however, making it experimentally very difficult to investigate the role of the components giving rise to them, or to attribute them to a specific chloroplast component. In all preparations in which we have observed these signals they show strong orientation effects, similar to those observed in EPR studies of single crystals<sup>10</sup>. Fig. 2 shows the effect of rotating a dark sample (*a* in Fig. 1) in the EPR cavity. If the sample is oriented to give maximal signal size ( $0^\circ$ ) and then rotated the signal disappears ( $40^\circ$ ) and then reappears ( $180^\circ$ ). At intermediate positions the signal size, linewidth and  $g$  value differ. Figure 2c ( $50^\circ$ ) shows this effect. We have found that in order to be sure that a sample does or does not have a signal the spectrum must be recorded every  $10^\circ$  through  $180^\circ$ , 18 spectra per sample. The spectra in Fig. 1 show the maximum signal size for 0, 1, 2, 3 and 4 flashes from a set of 18 spectra for each sample. The apparent  $g$  value also changes with orientation as is observed in a single crystal. The  $g$  value and linewidth of the dark signal is also temperature dependent. Figure 3 shows the temperature dependence of the dark signal. The signal has a higher  $g$  value at lower temperatures with  $g = 3.38$  at 3.8 K,  $g = 3.27$  at 11.7 K and  $g = 3.14$  at 19.8 K. The linewidth also changes with temperature becoming narrower at lower temperatures being 70 G at 3.8 K, 120 G at 11.7 K and 170 G at 19.8 K with the sample orientation used in Fig. 3. The temperature dependence of the  $g$  value of the dark signal suggests that the signal arises from a ferromagnetic complex. Ferromagnetism is most commonly observed with iron; however, several other transition metals, but not apparently

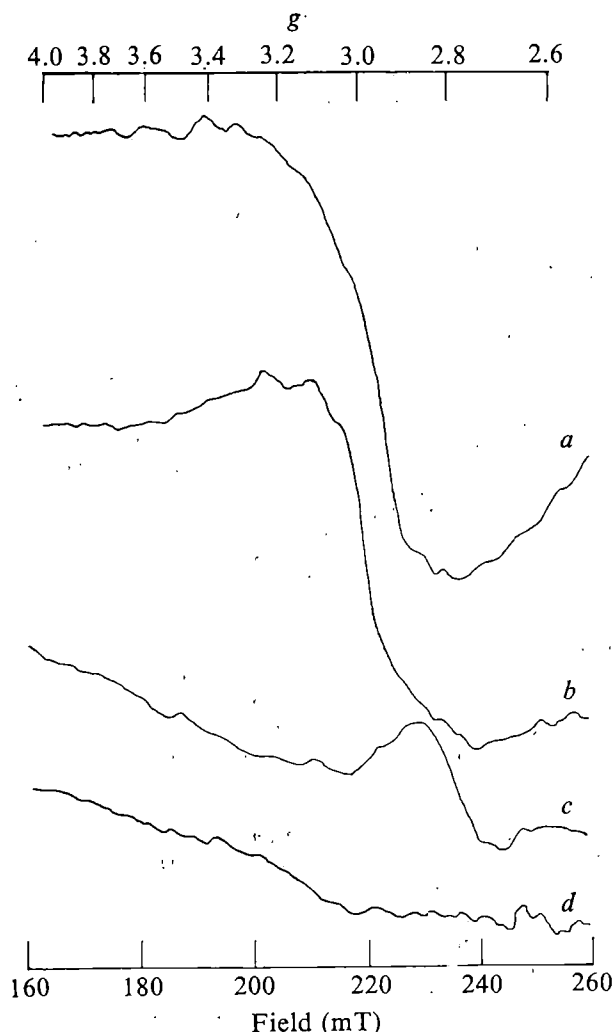


Fig. 2 Effect of sample position on the dark EPR signal in broken spinach chloroplasts. Sample and conditions as for Fig. 1a. The relative orientations of the samples were *a*,  $0^\circ$ ; *b*,  $180^\circ$ ; *c*,  $50^\circ$  and *d*,  $40^\circ$ .

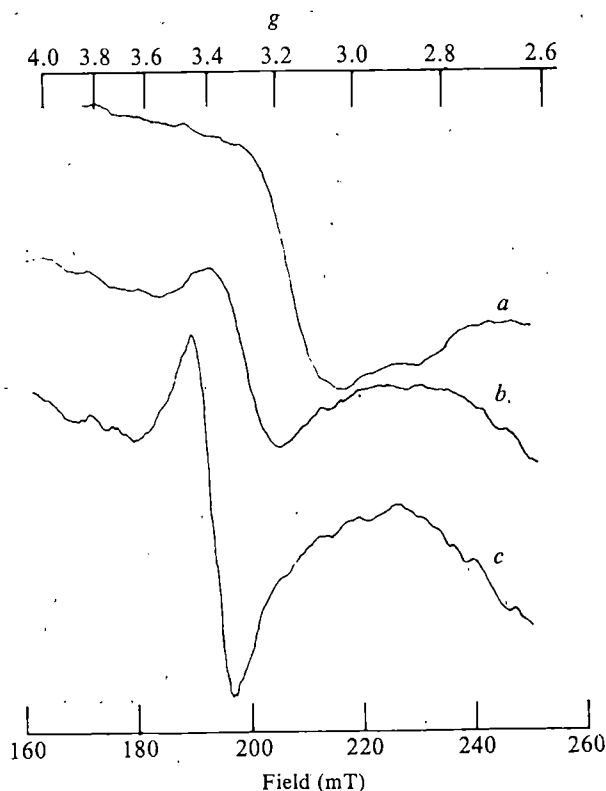


Fig. 3 Effect of temperature on the dark EPR signal in broken spinach chloroplasts. Conditions and samples as in Fig. 1a except that the temperatures were *a*, 19.8 K; *b*, 11.7 K; *c*, 3.8 K.

manganese, can show this property<sup>11</sup>. Ferromagnetism is a co-operative effect seen only when two or more atoms are in close proximity. Oriented ferromagnetic EPR signals have been reported from nerve tissue and nucleic acid preparations<sup>12,13</sup>. These signals were observed at room temperature, however, unlike the signals reported here which could not be observed above 40 K. We have not observed any temperature dependence of the  $g$  value of the signal seen after two flashes, indicating that the redox state giving rise to this signal is not ferromagnetic and is therefore different from the state giving rise to the dark signal.

Although the properties of these signals are unusual, the results presented here have been obtained consistently mainly in spinach chloroplasts but also in lettuce and pea chloroplasts. They are not seen in chloroplasts which have been washed with Tris, (0.8 M Tris-HCl pH 8.0 for 10 min at  $2^\circ$ , the thylakoids were then centrifuged and resuspended in the original medium), a procedure which inactivates the water oxidation system<sup>14</sup>, or in photosystem I particles prepared with the anionic detergent Triton X-100. The association of these signals with chloroplasts and their absence from photosystem I particles, together with the observation that they are lost when the oxygen evolving system is destroyed, suggest that the signals are associated with photosystem II. EPR signals responding to single turnover flash illumination might be associated with either the electron donor or acceptor sides of photosystem II. But signals associated with the acceptor would be expected to show a binary, two flash, response cycle, as the acceptor is thought to be a quinone which would be fully reduced in two steps. Signals associated with the water-oxidising system would be expected to show a four-flash response cycle. The signals reported here apparently show a four flash cycle as two states with different signals and two states without signals are observed. These changes would correspond to a model of the oxygen-evolving complex in which the electrons are removed sequentially from a single complex. They would not fit models in which electrons are removed from isolated centres each of which undergoes the same redox reaction.

We conclude that these signals probably reflect the oxidation state of the water oxidising enzyme system; future experiments

should lead to the identification of the component giving rise to these signals and the properties of this component which lead to orientation of the signal.

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1. Chaeninae, G. M. A. *Rev. Pl. Physiol.* 21, 467-498 (1970).
2. Joliot, P., Barbieri, G. & Chaband, R. *Photochem. Photobiol.* 30, 309-329 (1969).
3. Kok, B., Forbush, B. & McGloin, M. *Photochem. Photobiol.* 11, 457-475 (1970).
4. Radmer, R. & Kok, B. A. *Rev. Biochem.* 44, 409-433 (1975).
5. Bolton, J. R. & Warden, J. T. A. *Rev. Pl. Physiol.* 27, 375-383 (1976).
6. Pulles, M. P. J., Van Gorkom, H. J. & Willemsen, J. G. *Biochim. biophys. Acta* 449, 536-540 (1976).
7. Mathis, P. & Haveman, J. *Biochim. biophys. Acta* 461, 167-181 (1977).
8. Wydrzynski, T., Zumbulyadis, N., Schmidt, P. G., Gutowsky, H. S. & Govindjee *Proc. natn. Acad. Sci. U.S.A.* 73, 1196-1198 (1976).
9. Wydrzynski, T., Zumbulyadis, N., Schmidt, P. G. & Govindjee *Biochim. biophys. Acta* 408, 349-354 (1975).
10. Walker, S. & Straw, H. *Spectroscopy* 1, 176-178 (Chapman and Hall, London, 1969).
11. Bleaney, B. I. & Bleaney, B. *Electricity and Magnetism*, 2nd edn (Clarendon, Oxford, 1965).
12. Commoner, B., Woolam, J. C. & Larsson *Science* 165, 703-704 (1969).
13. Walsh, M. W. Jr, Shulman, R. G. & Heidenreich, R. D. *Nature* 192, 1041-1043 (1961).
14. Yamashita, T. & Tsuji, J. *Pl. Cell Physiol.* 12, 117-126 (1971).
15. Walker, D. A. & Slabas, A. R. *Pl. Physiol.* 57, 203-208 (1976).
16. Lilley, R. McC., Fitzgerald, M. P., Rienits, K. G. & Walker, D. A. *New Phytol.* 75, 1-10 (1975).
17. Evans, M. C. W., Telfer, A. & Lord, A. V. *Biochim. biophys. Acta* 267, 530-537 (1972).
18. Evans, M. C. W. & Cammack, R. *Biochem. biophys. Res. Commun.* 63, 1871-93 (1975).

## Effects of foetal haemoglobin on susceptibility of red cells to *Plasmodium falciparum*

HIGH gene frequencies for the sickling disorders and  $\beta$  thalassaemia may be due to the relative protection against *Plasmodium falciparum* malaria which has been afforded to heterozygous carriers<sup>1</sup>. In sickling disorders the properties of the abnormal haemoglobin may be responsible for this phenomenon<sup>2-4</sup> but the red cells of adult  $\beta$  thalassaemia heterozygotes contain reduced amounts of normal haemoglobin<sup>5</sup> and are indistinguishable from normal red cells with respect to the rates of invasion and growth of *P. falciparum* (G.P., D.J.W. and R.J.M., in preparation). Hence it is not clear how the  $\beta$  thalassaemia polymorphism has been maintained. There is a possible explanation, however. At birth red cells contain mainly foetal haemoglobin (Hb F); adult haemoglobin (Hb A) replaces Hb F during the first year of life. The rate of decline of Hb F production during this period is retarded in infants heterozygous for  $\beta$  thalassaemia as compared with normal infants<sup>6</sup>. If the presence of Hb F were to protect red cells against *P. falciparum*,  $\beta$  thalassaemia carriers aged 6-18 months might be at an advantage when passive immunity to *P. falciparum* is waning but active immunity is not fully established and mortality from malaria is high<sup>7</sup>. To test this hypothesis we have compared the rates of invasion and growth of *P. falciparum* in red cells containing Hb A with those containing Hb F using a modification of the *in vitro* culture system<sup>8-10</sup>.

Samples of 5 ml of blood containing synchronous late ring stages of *P. falciparum* were collected into heparin (10 IU ml<sup>-1</sup>) and layered onto a 6 × 2 cm column of Whatman CF11 cellulose in phosphate-buffered saline (PBS). The

leukocyte-depleted red cells were washed through the column into 2-l Ehrlenmeyer flasks with 100 ml supplemented medium 199<sup>8</sup>. The flasks were gassed with 5% CO<sub>2</sub>-95% air and incubated at 37 °C with gentle shaking. After about 24 h, when the parasites had grown to the schizont stage, the red cells were recovered by centrifugation and reconstituted to the original 5 ml volume with medium 199. Plasmagel 2.5 ml (3% w/v gelatin; Bellon) was added and the cells were allowed to sediment for 15 min at 37 °C to concentrate the schizont-infected cells in the upper layer. The latter were then suspended in a mixture of 3 parts supplemented medium 199 and 1 part non-immune AB serum. The uninfected red cells which were to be examined for the rate of parasite invasion and growth were collected into heparin, washed three times in PBS, the buffy coat removed and added to an appropriate volume of the parasitised red cell suspension so that the final concentration was approximately 80 × 10<sup>3</sup> cells per  $\mu$ l with 2-5% schizont-infected cells. The mixture was plated into the wells of a microtissue culture plate (250  $\mu$ l per well) and incubated in 5% CO<sub>2</sub>-air at 37 °C without shaking. At various times blood films were made and either stained with Giemsa or treated by the acid-elution method<sup>11-13</sup> modified to demonstrate Hb F-containing cells while at the same time allowing adequate morphological characterisation of parasites.

*P. falciparum* preferentially invades young, metabolically active red cells<sup>14</sup>. As this study was designed to compare the rates of invasion and growth *in vitro* in cells containing Hb A with those containing Hb F, and as these cells might be of different metabolic ages, a preliminary series of experiments was carried out to examine the effect of cell age on the rates of invasion and growth of *P. falciparum* in the *in vitro* system. Normal red cells were fractionated by age<sup>15,16</sup> and each fraction was mixed separately with infected cells. In each case the rate of invasion of the younger red cell population was significantly greater than that of the

Table 1 Invasion and development of *P. falciparum* in normal red cells of different ages

Cell fraction	Reticulocytes (%)	Invasion Parasites (%)	Developmental stages of 100 parasites per cell fraction							
			R		S		L		G	
			S	L	S	L	S	L	S	L
Whole blood	0.4	5.0	0	3	40	30	11	1	2	13
Top	2.5	7.9	0	2	39	32	11	2	1	13
Middle	0.6	3.6	0	4	36	29	14	3	0	14
Bottom	0.2	3.3	0	3	36	24	16	1	2	18
Whole blood	2.4	5.1	0	1	31	37	14	1	1	15
Top	9.6	8.9	0	3	40	26	10	2	0	19
Middle	1.5	4.5	0	2	42	24	10	2	0	20
Bottom	0.1	3.2	0	0	49	21	10	1	0	19

Uninfected adult red cells were centrifuged at 2,500g for 1 h at 4 °C. Fractions of approximately 0.2 ml were removed from the top, middle and bottom of the column of red cells and from whole blood. Reticulocyte counts were done to confirm separation on the basis of age. Suspensions of schizont-infected cells were added to each fraction and the mixtures were incubated as described in the text. The percentage of ring forms in each fraction arising from a fixed inoculum of schizonts is shown for two experiments. At least 1,000 red cells were examined from each fraction. In each case a  $\chi^2$  test showed a significantly greater percentage of parasites in the top (young) cell as compared with the bottom fraction ( $P < 0.01$ ). To assess parasite development smears were made after 53 h in culture and the maturity of parasites in 100 singly infected cells in each fraction was assessed. Rings (R) were forms without pigment, trophozoites (T) had a single clump of pigment and a single nucleus, schizonts (S) had more than one nucleus, gametocytes (G) had diffuse pigment, and abnormal forms (A) were those in which there was no nuclear-cytoplasmic differentiation on staining and which were pyknotic. The rings, trophozoites and schizonts were further subdivided into small (S) and large (L) forms. There was no significant difference in the pattern of development of the parasites between the various cell fractions.



older cells (Table 1). The further growth and development of the parasites in the different aged cell populations were assessed both by morphological examination (Table 1) and  $^3\text{H}$ -isoleucine incorporation<sup>17,18</sup>. Parasite growth assessed by either method was similar in each cell fraction. Thus in this *in vitro* system the parasites preferentially invade young metabolically active cells but there is virtually no difference in the rates of development in red cells of differing ages.

To determine whether Hb F has any effect on invasion of red cells by *P. falciparum*, the relative rates of invasion of Hb A and Hb F-containing cells were compared. Hb A-containing cells were obtained from normal adults. Hb F-containing cells were derived from four sources—normal newborn infants, infants aged 3–6 months, homozygotes for the British type of hereditary persistence of foetal haemoglobin (HPFH)<sup>19</sup> and heterozygotes for the African form of HPFH<sup>20</sup>. The choice of these Hb F-containing cells was based on the following rationale. The red cells of newborn infants contain mainly Hb F and are metabolically younger than those of normal adults<sup>21</sup>; by making an artificial mixture of newborn-infant and adult cells it would be possible to compare the rates of invasion of two red cell populations of different metabolic ages with Hb F in the 'younger' cells. In the blood of infants aged 3–6 months Hb F production has almost ceased and the newly-produced red cells contain almost entirely Hb A; in the same blood sample it would be possible to examine the relative rates of invasion of Hb A and Hb F-containing cells, in this case the latter being the metabolically older population. The red cells of homozygotes for the British form of HPFH contain increased amounts of Hb F which is unevenly distributed, some cells carrying mainly Hb F and others mainly Hb A<sup>18</sup>. Since the metabolic ages of these populations are similar it would be possible to study the relative invasion rates of Hb F and Hb A-containing cell populations of the same metabolic age in the same blood sample. The red cells in the African form of HPFH, apart from their increased levels of Hb F, are similar in

every way to those of normal adults<sup>19</sup>. The results are summarised in Table 2. In the mixture of newborn and adult red cells the Hb F-containing cells were preferentially invaded, in the red cells of the 3–6-month old infants there were significantly more parasites in the Hb A-containing cells, and in the red cells of the British HPFH homozygotes the Hb F and Hb A-containing populations were equally invaded. The rates of invasion of the African HPFH cells were identical to those of normal adult cells in parallel cultures. These experiments show that Hb F has no direct effect on the rate of invasion of red cells by *P. falciparum* and provide further evidence that invasion is a cell-age related process.

**Table 3** Development of *P. falciparum* in cells containing varying amounts of foetal haemoglobin

Blood type	Cell type	Developmental stages of 100 parasites per group							
		R		T		S		G	A
		S	L	S	L	S	L		
Cord	A	0	0	25	58	8	5	2	2
	F	0	3	64	23	3	0	2	5
	A	0	1	29	48	17	2	0	3
	F	0	3	78	14	0	0	0	5
$\beta^0/\delta\beta$ Thalassaemia	A	8	10	44	28	6	0	0	4
	F	34	29	25	9	0	0	0	3
	A	10	30	25	27	7	0	0	1
	F	42	38	17	1	0	0	0	2
British HPFH	A	3	21	31	34	8	0	1	2
	I	14	26	28	20	6	0	0	6
	F	26	10	27	18	1	0	0	18
	A	21	5	4	8	30	19	2	1
	I	45	14	4	11	20	3	0	3
	F	46	5	8	17	13	7	0	4
African HPFH	A	1	6	27	20	19	9	0	18
	F	1	10	33	20	9	3	0	24
	A	0	3	45	34	7	1	0	10
	F	0	4	54	24	1	0	1	16
Infant	A	21	43	14	9	11	3	0	0
	I	22	68	8	0	2	0	0	0
	F	24	67	7	2	0	0	0	0
	A	10	31	33	12	9	0	0	5
	I	30	30	28	8	0	0	0	4
	F	28	34	28	4	0	0	0	6

Experimental methods are described in the text and the designation of cell type and the development stages of the parasites are the same as those in Table 1. All cells were cultured in the same wells except for the African HPFH and Hb A-containing control cells which were in parallel. There is a significant retardation of development of parasites in the Hb F-containing cells in each case.

A further series of experiments was carried out to compare the development of *P. falciparum* in Hb F-containing cells with that in cells which contain mainly Hb A. In addition to the cells used in the experiments described above a mixture of normal adult cells and cells from a compound  $\beta^0/\delta\beta$  thalassaemia heterozygote which contain almost 100% Hb F<sup>22</sup> were studied. The cells were infected *in vitro* and at various times smears were made from the cultures and a comparison of the maturity of parasites in the Hb A and Hb F-containing cells was made. The results of representative experiments are summarised in Table 3. There was a significant reduction in the number of mature forms of *P. falciparum* in the Hb F-containing cells regardless of their source or age. Hence the presence of Hb F in red cells causes retardation of growth of *P. falciparum* *in vitro*. Since it was observed in red cells containing Hb F obtained from both infants and adults with several different genetic conditions, this phenomenon is probably related to

**Table 2** Invasion by *P. falciparum* of red cells containing varying amounts of foetal haemoglobin

Cell type	Adult+cord		Source of Hb F-containing cells				Infant	
			Parasites	F/A	Parasites	F/A	Parasites	F/A
			(%)		(%)		(%)	
A	5.2		10.8		3.1		7.4	
I	—	2.3	8.9	0.9	—	1.0	2.9	0.3
F	12.1		10.1		3.0		2.4	
A	5.1		9.6		7.8		4.3	
I	—	2.6	9.0	1.1	—	0.9	1.6	0.2
F	13.2		10.2		6.7		0.8	
A	5.5		4.3		6.2		12.2	
I	—	1.8	4.9	1.1	—	1.0	7.0	0.5
F	9.9		4.9		6.1		5.8	

Experimental conditions as described in the text. Where Hb A and Hb F-containing cells were present in the same culture, smears were stained by a modification of the acid elution technique to distinguish between the following groups of cells:— those containing predominantly Hb A where the haemoglobin was completely eluted (A), cells containing Hbs A and F (intermediate or I cells) and cells containing mainly Hb F (F cells)<sup>13</sup>. Since the mixture of adult and cord cells was artificial the group of I cells has been omitted. The African HPFH and adult control cells were grown in parallel and not in the same wells, as the acid elution technique does not readily distinguish African HPFH cells from those containing Hb A in an artificial mixture. In these cases the intermediate (I) group is omitted. At least 2,000 cells were counted in each experiment. The difference in parasitisation of the A and F cells in the adult/cord and infant cell experiments was significant at the 1% level. There was no significant difference between the parasite counts in the A and F populations in the British or African HPFH red cells.

the presence of Hb F and not to other metabolic properties of the red cells. Further evidence that this is the case was obtained in those experiments in which it was possible to define red cells which contained intermediate amounts of Hb F (Table 3). The degree of retardation of growth of *P. falciparum* in these cells was approximately intermediate between that in cells which contained predominantly Hb A or Hb F suggesting that the degree of retardation of growth of the parasite is broadly correlated with the level of Hb F in the red cell. Since the phenomenon was observed in the red cells of the African HPFH heterozygote, it seems likely that growth retardation can result from as little as 7 pg Hb F per cell.

Whether Hb F causes relative retardation of growth and development of *P. falciparum* by its high oxygen affinity, different primary structure from that of Hb A, or by another mechanism, remains to be determined. Whatever the mechanism this phenomenon, taken together with the retardation of the rate of decline of Hb F production over the first year of life in infants heterozygous for  $\beta$  thalassaemia, offers a possible mechanism for the maintenance of the  $\beta$  thalassaemia polymorphism. Any property of the red cell which causes even minor growth retardation or interferes with synchronisation of development of the parasite might provide some degree of protection, particularly at a stage of development when passive immunity is waning and active immunity is not established. Further data on the levels of Hb F which are required for protection, and on the intracellular levels of Hb F in such heterozygous infants during their first year is required to test this hypothesis. Its particular attraction is that because the rate of decline of Hb F is retarded in infants with  $\beta$  chain structural haemoglobin variants as well as those with  $\beta$  thalassaemia<sup>8</sup>, it provides a unifying mechanism for the high gene frequencies of the  $\beta$ -chain haemoglobinopathies. It does not, of course, exclude the operation of other protective mechanisms such as have been proposed for cells containing Hb S<sup>9-11</sup>.

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- Allison, A. C. in *Abnormal Haemoglobins in Africa* (ed. Jonxis, J. H. P.) 365-392 (Blackwell, Oxford, 1965).
- Allison, A. C. *Br. med. J.* 1, 290-294 (1954).
- Luzzatto, L., Nwachuku Jarrett, E. S. & Reddy, S. *Lancet* 1, 319-321 (1970).
- Luzzatto, L. *Bull. Wld Hlth. Org.* 50, 195-202 (1974).
- Weatherall, D. J. & Clegg, J. B. in *The Thalassaemia Syndromes* (Blackwell, Oxford, 1972).
- Beaven, G. H., Ellis, M. J. & White, J. C. *Br. J. Haemat.* 7, 169-185 (1961).
- McGregor, I. A., Williams, K., Voller, A. & Billewicz, W. Z. *Trans. R. Soc. trop. Med. Hyg.* 59, 395-414 (1965).
- Phillips, R. S., Trigg, P. I., Scott-Finnegan, T. J. & Bartholomew, R. K. *Parasitology* 65, 525-535 (1972).
- Phillips, R. S., Rahman, A. K. & Wilson, R. J. M. *Trans. R. Soc. trop. Med. Hyg.* 69, 432 (1975).
- Trigg, P. I. *Bull. Wld. Hlth. Org.* 53, 399-406 (1976).
- Kleihauer, E., Braun, H. & Betke, K. *Klin. Wschr.* 35, 637-638 (1957).
- Diggs, C. *et al. J. Parasit.* 57, 185-186 (1971).
- Pasvol, G., Weatherall, D. J., Wilson, R. J. M., Smith, D. H. & Gilles, H. M. *Lancet* 1, 1269-1272 (1976).
- Wilson, R. J. M., Pasvol, G. & Weatherall, D. J. *Bull. Wld Hlth. Org.* (in the press).
- Borun, E. R., Figueroa, W. G. & Perry, S. M. *J. clin. Invest.* 36, 676-679 (1957).
- Pranker, T. A. *J. Physiol., Lond.* 143, 325-331 (1958).
- McCormick, G. J. *Expl Parasit.* 27, 143-149 (1970).
- Trigg, P. I. & Gutteridge, W. E. *Parasitology* 62, 113-123 (1971).
- Weatherall, D. J. *et al. Br. J. Haemat.* 29, 205-220 (1975).
- Conley, C. L., Weatherall, D. J., Richardson, S. N., Shepard, M. K. & Charache, S. *Blood* 21, 261-281 (1963).
- Keitt, A. S. in *Hematology of Infancy and Childhood* (eds Nathan, D. G. & Oski, F. A.) 23 (Saunders, Philadelphia, 1974).
- Ottolenghi, S. *et al. Proc. natn. Acad. Sci. U.S.A.* 72, 2294-2299 (1975).

## Further link between complement activation and blood coagulation

EVIDENCE for interactions between the complement and haemostatic systems has come from two lines of research—blood platelets have been shown to interact with various complement components<sup>1-6</sup>, and more ambiguous results have been obtained with respect to the role of complement in endotoxin shock and the Schwartzman reaction<sup>7-13</sup>. We report here that the activated complement component C3b triggers a marked increase of tissue thromboplastin (factor III) activity in cultured human monocytes. Differential counting and non-specific esterase staining<sup>14</sup> of the final preparations regularly revealed more than 85% monocytes.

After isolation and incubation as described in Table 1, the cells were rinsed 10 times with sterile saline and mechanically displaced with a rubber policeman into 1 ml of Veronal-buffered saline<sup>15</sup>. Tissue thromboplastin activity was tested as before<sup>16</sup>. Procoagulant activity required the presence of coagulation factor VII and was inactivated rapidly by phospholipase C<sup>17</sup> and by a monospecific antiserum against tissue thromboplastin<sup>18,19</sup>. The procoagulant was thus identified as tissue thromboplastin. Guinea pig complement factor C3b was generated and purified as described by Nicholson *et al.*<sup>20</sup>.

Table 1 summarises data from five experiments. C3b gives a marked increase in tissue thromboplastin activity of isolated monocytes cultured in the presence or absence of serum. This increase was of the same order of magnitude as that seen after endotoxin addition (data not shown). Cycloheximide (Sigma) (final concentration 10-20  $\mu$ g ml<sup>-1</sup>) inhibited the increase completely; thus protein synthesis was probably required. The effect of C3b was significant even at the lowest concentration

Table 1 Effect of C3b on tissue thromboplastin activity of human monocytes

Experiment	Additions ( $\mu$ g ml <sup>-1</sup> )	Serum (20%)	Activity (units per mg protein)	Increase* (%)
1	C3b (0.5)	+	41.7	256
	C3b (0.5) + cycloheximide (20)	+	11.7	0
	None (control)	+	11.7	
2	C3b (12.5)	+	37.0	348
	C3b (12.5)	-	18.7	127
	None (control)	+	8.2	
3	C3b (15)	+	20.6	131
	C3b (30)	+	25.7	189
	None (control)	+	8.9	
4	C3b (30)	-	15.5	78
	None (control)	-	8.7	
5	C3b (30) + endotoxin (50)	+	69.3	1,055
	C3b (30)	+	56.6	843
	None (control)	+	6.0	

Human monocytes were isolated from mixed buffy coats by a modification of the Bøyum method<sup>21</sup> including dextran sedimentation, two Ficoll-Paque (Pharmacia), gradients and several differential centrifugations. The cells adhering to the Petri dishes (Nunc), were selected by washing away non-adherent cells. Incubations lasted for 5 h. A standard preparation of human brain thromboplastin that clotted normal citrated plasma in 13 s was taken to contain 100 units ml<sup>-1</sup> and dilutions of this preparation were used to establish a standard curve. Protein was determined by the Lowry method<sup>22</sup>. The endotoxin was *Escherichia coli* 055:135 type B (Difco). Each value is the mean of determinations in triplicate on duplicate cultures. The test samples had protein concentrations of 117-726  $\mu$ g ml<sup>-1</sup> and the actual clotting times recorded were 18.7-38.8 s in C3b-stimulated samples and 41.3-68.8 s in controls. The clotting times were converted to thromboplastin units by means of the standard curve and normalised to units per mg protein.

\*Control values subtracted.



tested ( $0.5 \mu\text{g ml}^{-1}$ ). The apparent lack of a dose-response relationship is caused by the varying magnitude of the response of the different cell preparations used in the various experiments. In experiment 3 (Table 1) where different aliquots of the same cell preparation were exposed to two different levels of C3b, the higher dose gave the higher response. Unstimulated monocytes cultured for 5 h had very low activities (Table 1). C3b alone did not influence the assay system (tested up to  $30 \mu\text{g ml}^{-1}$ ).

Isolated monocytes develop increased procoagulant activity when stimulated by endotoxin<sup>23</sup>. This phenomenon probably requires both mRNA and protein synthesis (H.P. and A.C.A., in preparation) and is seen in monocyte cultures virtually free from blood platelets. Granulocytes and non-adherent mononuclear cells are essentially unresponsive (data not shown).

Human monocytes and other macrophages have receptors for C3b<sup>24,25</sup>, and C3b is known to induce release of lysosomal enzymes from macrophages<sup>26</sup>. The production of C3 by normal macrophages<sup>27</sup> makes possible a positive feedback cycle<sup>28</sup>. This link between complement activation and the extrinsic blood coagulation system has pathogenetic implications in the initiation of disseminated intravascular coagulation and possibly of thrombosis, which are under study.

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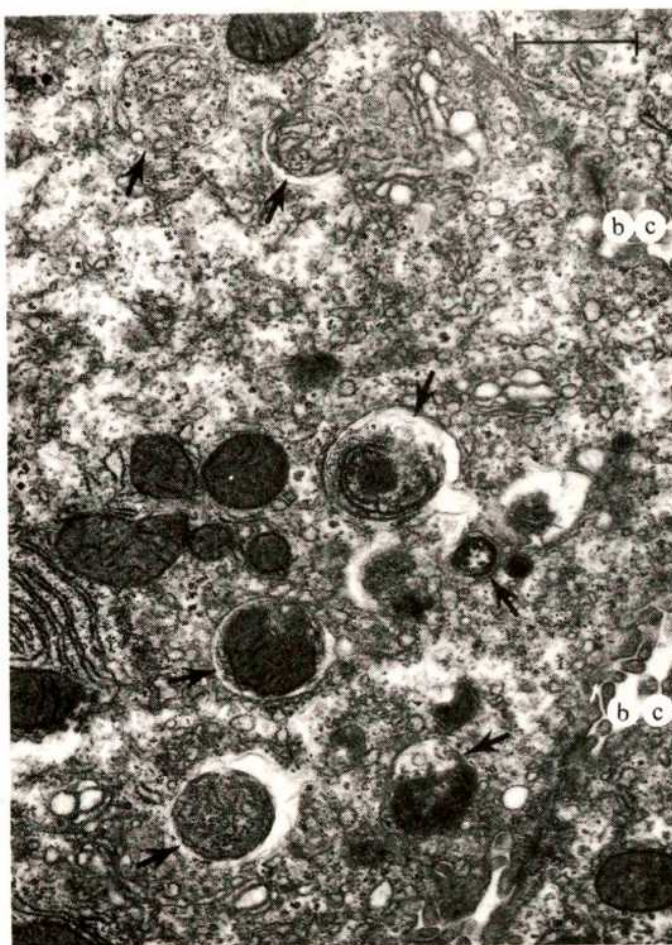
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- Wautier, J. L., Tobelem, G. M., Peltier, A. P. & Caen, J. P. *Haemostasis* 2, 281-286 (1973/74).
- Wautier, J. L., Souchon, H., Cohen Solal, L., Peltier, A. P. & Caen, J. P. *Immunology* 31, 595-599 (1976).
- Cazenave, J.-P., Assimeh, S. N., Painter, R. H., Packham, M. A. & Mustard, J. F. *J. Immunol.* 116, 162-163 (1976).
- Suba, E. A. & Csako, G. J. *Immunol.* 117, 304-309 (1976).
- Breckenridge, R. T., Rosenfeld, S. L., Graff, K. S. & Leddy, J. P. *J. Immunol.* 118, 12-16 (1977).
- Zimmerman, T. S. & Kolb, W. P. *J. Immunol.* 116, 1755 (1976).
- Brown, D. L. & Lachmann, P. J. *Int. Arch. Allergy* 45, 193-205 (1973).
- Polák, L. & Turk, J. L. *Nature* 223, 738-739 (1969).
- Garner, R., Chater, B. V. & Brown, D. L. *Br. J. Haemat.* 28, 393-401 (1974).
- Müller-Berghaus, G. & Lohmann, E. *Br. J. Haemat.* 28, 403-417 (1974).
- Ulevitch, R. J., Cochrane, C. G., Henson, P. M., Morrison, D. C. & Doe, W. F. *J. exp. Med.* 142, 1570-1590 (1975).
- Evensen, S. A., Pickering, R. J., Batboute, J. & Shepro, D. *Eur. J. clin. Invest.* 5, 463-469 (1975).
- Gilbert, V. E. & Braude, A. I. *Fedn Proc.* 21, 17 (1962).
- Yam, L. T., Li, C. Y. & Crosby, W. H. *Am. J. clin. Path.* 55, 283-290 (1971).
- Hjort, P. F. *Scand. J. clin. Lab. Invest.* 9, suppl. 27 (1957).
- Uvattum, M. & Prydz, H. *Biochim. biophys. Acta* 130, 92-101 (1966).
- Otnaess, A. B., Prydz, H., Bjørklid, E. & Berre, Å. *Eur. J. Biochem.* 27, 238-243 (1972).
- Bjørklid, E., Storm-Mathisen, J., Storm, E. & Prydz, H. *Thrombos. Haemostas.* 37, 91-97 (1977).
- Bjørklid, E. & Storm, E. *Biochem. J.* (in the press).
- Nicholson, A. et al. *J. Immunol.* 115, 1108-1113 (1975).
- Boyum, A., *Scand. J. clin. Lab. Invest.* 21, 77-89 (1968).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* 193, 265-275 (1951).
- Rivers, R. P. A., Hathaway, W. E. & Weston, W. L. *Br. J. Haemat.* 30, 311-316 (1975).
- Lay, W. H. & Nuzzenzweig, V. *J. exp. Med.* 128, 991-1009 (1968).
- Huber, H., Polley, M. J., Luiscott, W. D., Fudenberg, H. H. & Müller-Eberhard, H. J. *Science* 162, 1281-1283 (1968).
- Schorlemmer, H. U., Davies, P. & Allison, A. C. *Nature* 261, 48-49 (1976).
- Lai A Fat, R. F. M. & Van Furth, R. *Immunology* 28, 359-368 (1975).
- Ferluga, J., Schorlemmer, H. U. & Allison, A. C. *Clin. exp. Immunol.* (in the press).

## Induction of autophagy by amino-acid deprivation in perfused rat liver

AUTOPHAGOCYTOSIS is generally regarded as an important mechanism for ridding the cell of injured or unwanted cytoplasmic constituents and for degrading normal components in response to energy needs<sup>1,2</sup>. Autophagy has been noted in HeLa cells deprived of foetal calf serum and amino acids<sup>3</sup>. It

has also been seen in rat liver after glucagon administration<sup>4,5</sup>, during nutritional deprivation<sup>7,8</sup> and in diabetes<sup>9,10</sup>. Thus autophagy could have a physiological role in the maintenance of body amino acid pools, although there is no direct experimental evidence for this. We reported previously that rates of proteolysis, and lysosomal sizes are seen to increase when rat livers are cyclically perfused with an unsupplemented medium and decrease in response to added amino acids<sup>11-13</sup>. We strongly suspected that autophagy was the underlying event, but the nature of the process has been difficult to define<sup>12</sup>. The possibility was considered that its expression was self-limited by the accumulation of amino acids from endogenous proteo-



**Fig. 1** Electron micrograph of a portion of a hepatocyte, adjacent to two bile capillaries (bc), from a liver perfused in the single-pass mode for 40 min with medium not supplemented with amino acids. After perfusion, livers were flushed with 10 ml of cooled perfusate plasma followed by 40 ml (in 4 min) of Karnovsky's glutaraldehyde-paraformaldehyde fixative<sup>2</sup>, diluted 1:7 with 0.1 M sodium cacodylate (pH 7.2); 1-mm cubes then were cut from the major lobes and fixation continued with full-strength Karnovsky's medium for 3-4 h in an ice bath; the tissues were post-fixed in 1.33% osmium tetroxide, buffered with s-collidine (pH 7.4) for 2 h at ice temperature. The blocks were imbedded in epoxy resin (Durcupan ACM, Fluka) and thin sections cut with a Sorvall Porter-Blum MT 2-B ultramicrotome from an area containing predominantly mid-zonal cells<sup>25</sup>. The sections were double-stained with uranyl acetate and either lead citrate or lead hydroxide before examination with an RCA-EMU-IV electron microscope. Numerous autophagic vacuoles are evident (arrows). Smooth endoplasmic reticulum, glycogen, a mitochondrion, and a portion of rough endoplasmic reticulum are especially well preserved within the vacuoles. Several of the autophagic vacuoles are limited by a double membrane throughout a significant portion of their circumference. In general, the hepatocytes seem to be in good condition. Mitochondrial swelling is not evident nor are there any large cytoplasmic vacuoles. There is some vesiculation which probably represents enlarged elements of the Golgi complex and which may play a part in the autophagic process.



lysis. We now show that when this accumulation is prevented by perfusing livers in the single-pass mode, autophagic changes appear that are morphologically indistinguishable from those occurring after maximal doses of glucagon<sup>4-6</sup>.

Livers from fed male rats of the Lewis strain (Microbiological Associates), weighing 120–150 g, were perfused *in situ* with a medium consisting of freshly washed bovine or sheep red cells suspended in Krebs–Ringer bicarbonate buffer (0.27 v/v) containing 3% bovine plasma albumin (Pentex) and 10 mM glucose. Amino acid levels in the perfusate plasma were reduced to 2.5% of normal rat plasma values as determined by direct analysis<sup>14</sup>. Perfusions were carried out as described previously<sup>15</sup> except that in all single-pass runs the outflow from the livers was not returned to the perfusion flasks<sup>16</sup>.

Perfusion of livers in the single-pass mode with a medium depleted of amino acids evoked a striking increase in autophagy (Figs 1 and 2) that attained maximal intensity by 20 min and was clearly evident as early as 5 min (data not shown). Although autophagic vacuoles were widely scattered throughout the cell, they were more numerous in the vicinity of Golgi vesicles. The variety of identifiable cytoplasmic inclusions was fairly typical of the contents of glucagon-induced vacuoles<sup>17</sup>. The one exception was glycogen; while it was a frequent lysosomal constituent in our experiments, it has not been noted consistently in glucagon studies<sup>4-6,17</sup>. This difference undoubtedly reflects the glycogen content of the cell at the time autophagy was underway. In most of these reports, the animals were starved and residual glycogen was undoubtedly lost through the glycogenolytic action of glucagon (through cyclic AMP). Although amino acid deficiency *per se* does not, in our experience, enhance glycogenolysis, we, nevertheless, considered the possibility that the albumin used in the medium might have carried substances capable of stimulating adenyl cyclase and autophagy. Analysis of tissue samples showed that cyclic AMP was not elevated, however (Table 1).

Effects of amino acid deficiency on the fractional cytoplasmic volumes of typical autophagic vacuoles are given in Fig. 2. With maximal deprivation, the fractional volume of autophagic vacuoles, 1.6%, was considerably larger than peak values after glucagon<sup>18</sup>, but compared closely with estimates after diabetes<sup>19</sup> and in neonatal rat livers at the time of maximal autophagy<sup>18</sup>. In other respects, however, our findings are similar to those seen with glucagon. The cross-sectional dimensions of lysosomal profiles, which were up to 1.8  $\mu$ m (Fig. 3), are in basic agreement with the data of Deter *et al.*<sup>5</sup>—we also noted, as they did, a significant decrease in the fractional volume of typical dense bodies (shaded fraction, Fig. 2). The addition of a 10 $\times$  physiological mixture of amino acids during single-pass perfusions completely prevented this autophagic increase and even reduced values below those in unperfused liver (Fig. 2). When the amino acids were added after autophagy was fully manifest (20 min), the vacuoles regressed almost completely with a half-time of 8–9 min (data not shown). This agrees closely with the time required to reverse the enhancement of lysosomal osmotic sensitivity in the perfused rat liver ( $t_{1/2}$  ~ 8 min)<sup>19</sup>, and as a first approximation, suggests that the fractional turnover rate for mixed autophagic vacuole contents is of the order of 0.08 per min.

During cyclic liver perfusion, most amino acids quickly attain steady-state levels that are about the same or only slightly lower than normal plasma values, and a few (including leucine, isoleucine, valine, glutamic acid and glycine) continue to rise<sup>13</sup>. But, as there is no external supply to replace those that are metabolised, a moderate deficiency does in fact exist and extra amino acids are provided by a significant increase in the rate of cytoplasmic proteolysis. It is evident from Figs 2 and 3 that autophagy and lysosomal enlargement increase in these conditions although to a smaller extent than was observed after more stringent deprivation. Our previous failure to demonstrate typical autophagic alterations during cyclic perfusion<sup>12</sup> can probably be explained by the technique of fixation used. In subsequent work we have shown it to be

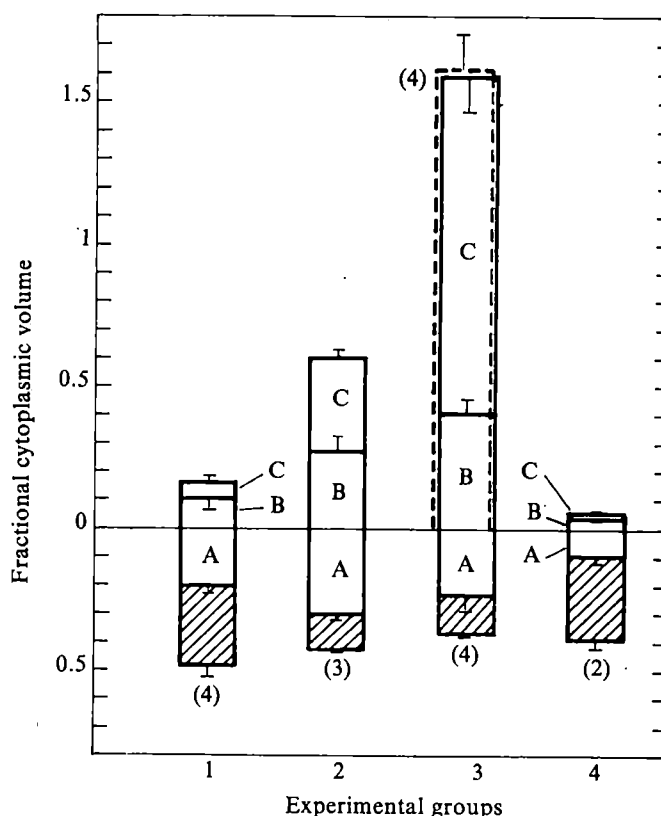


Fig. 2 Fractional cytoplasmic volume of components of the lysosomal-vacuolar system. The following experimental conditions were evaluated: (1), no perfusion; (2), cyclic perfusion for 60 min with no additions of glucose or amino-acids; (3), single-pass perfusion for 40 min with 10 mM glucose but no added amino acids (the offset dashed line represents values obtained from livers perfused 20 min in the same conditions); and (4), single-pass perfusion for 40 min with a medium supplemented with amino acids at a concentration 10 $\times$  normal plasma levels and 10 mM glucose. The components of the lysosomal-vacuolar system have been divided into four groups. Each bar represents mean  $\pm$  s.e.m., of hepatic cytoplasm that is occupied by the designated component. The organelles classically described as autophagic vacuoles<sup>4-6</sup> appear above the horizontal axis. These autophagic vacuoles have been divided into two types: type B contains only elements of smooth endoplasmic reticulum,  $\pm$  glycogen; the remainder were classified as type C. A significant proportion of the inclusions within type C were recognisable mitochondria, rough endoplasmic reticulum, or free ribosomes. In addition, type C vacuoles contained unrecognisable membrane remnants and amorphous material which were probably cytoplasmic constituents in various stages of intralysosomal digestion. The remainder of the lysosomal elements, which comprised the population of 'dense bodies' are shown below the horizontal axis of the graph. Many of these elements seemed identical to peribiliary dense bodies generally observed in normal liver tissue<sup>26,27</sup> (shaded area). Profiles were also observed which had some region of electron-dense material but also contained an area of granularity indicating the presence of glycogen. These profiles were designated type A autophagic vacuoles. Electron micrographs were analysed by the point-counting method<sup>28</sup> which involved a test grid of intersecting lines. The method of stratified random sampling<sup>29</sup> was used in selecting blocks of tissue from the two major lobes of the liver. Four to six micrographs were examined from each block of tissue. The number of livers in each experimental group is shown in parentheses. For comparison with fractional volumes expressed as % of whole liver volume, the values in this graph may be multiplied by 0.771, the fractional volume of hepatic cytoplasm with respect to the whole liver<sup>30</sup>.

less effective than the present method in preserving intralysosomal fine structure.

As shown in Fig. 2, lysosomal elements which contained no distinct membrane remnants were divided into two subgroups according to whether they (1) exhibited the usual electron opacity of dense bodies (shaded), or (2), contained additional sharply demarcated zones of electron lucency type A.



Table 1 Liver cyclic AMP levels

Experimental condition	Liver cyclic AMP (nmol per g liver)
Unperfused (9)	0.812±0.080
Perfused (8) no amino acids	0.973±0.184
Perfused (7) 10× amino acids	0.917±0.171

Liver perfusions were carried out in the single-pass mode for 40 min with or without amino-acids, as described in the text and in Fig. 2 legend. Value are means±s.e.m. The number of livers from each group is given in parentheses. Livers were rapidly frozen using Wollenberger clamps previously cooled in liquid nitrogen. Total cyclic AMP was determined using either the protein binding assay of Gilman<sup>31</sup> as modified by Fain *et al.*<sup>32</sup> or the procedure of Steiner *et al.*<sup>33</sup>.

In all instances, the lucent areas displayed the characteristic granulation pattern of glycogen; these bodies are presumed to be autophagic vacuoles containing glycogen and probably some associated cytoplasmic matrix and smooth endoplasmic reticulum. It is evident from Fig. 2 that the fractional cytoplasmic volume of type A lysosomes tended to increase with amino acid deprivation. It is also apparent from Fig. 2 that the composition of cytoplasmic elements within the larger, more typical vacuoles was not the same in the four conditions

studied. The proportion of large forms containing mitochondria and/or fragments of rough endoplasmic reticulum (type C) increased relative to the total autophagic fractional volumes (types B+C) as the level of autophagy increased. Thus sequestration does not seem to be a random process, but exhibits some selectivity according to the degree of amino-acid deprivation.

The effect of amino acid depletion on the sequestration of cytoplasmic constituents by the lysosomal-vacuolar system is seen at normal levels of cyclic AMP and suggests that hepatic autophagy is controlled by amino acids independently of tissue alterations of the cyclic nucleotide. Insulin may also suppress this process<sup>12</sup>, but it is clear that maximal inhibition can be obtained by amino acids alone. In view of the magnitude of this autophagic response, it probably could account for most or all of the increase in protein degradation that occurs with amino acid lack. Taking 0.08 per min as a minimal estimate of the fractional rate of turnover of cytoplasmic components (and of protein) within autophagic vacuoles, we have computed that the rate of cytoplasmic processing by type B and C autophagic vacuoles would be 6.0% per h per liver under stringent amino acid deprivation, 2.2% per h with moderate deprivation (cyclic perfusion), and 0.2% per h with no deprivation (amino acid additions). The difference between the last two estimates, 2.0% per h, is capable of accounting for the increase in hepatic proteolysis that occurs in the absence of added amino acids during cyclic perfusion<sup>12,13</sup>. Since the number of autophagic vacuoles in rat liver had been shown to rise sharply in the postabsorptive period<sup>7</sup>, autophagy could explain much of the loss of total liver protein during this period<sup>20</sup>. The same process might also account for the enhanced proteolysis observed in cultured eukaryotic cells during nutritional deprivation or in the absence of macromolecular growth substances<sup>21,23</sup>.

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- deDuve, C. & Wattiaux, R. *Ann. Rev. Physiol.* **28**, 435-492 (1966).
- Ericsson, J. L. E. in *Lysosomes* (eds Dingle, J. T. & Fell, H. B.) 345-394 (Elsevier, New York, 1969).
- Mitchener, J. S., Shelburne, J. D., Bradford, W. D. & Hawkins, K. C. *Am. J. Path.* **83**, 485-491 (1976).
- Ashford, T. P. & Porter, K. R. *J. Cell Biol.* **12**, 198-202 (1962).
- Deter, R. L., Baudhuin, P. & deDuve, C. *J. Cell Biol.* **35**, C11-C16 (1967).
- Arstila, A. U. & Trump, B. F. *Am. J. Path.* **53**, 687-733 (1968).
- Pfeifer, U. *Virchows Arch. B Zellpath.* **12**, 195-211 (1973).
- Swift, N. & Hruban, Z. *Fedn Proc.* **23**, 1026-1037 (1964).
- Pain, V. M., Lanoix, J., Bergeron, J. J. M. & Clemens, M. *Biochim. biophys. Acta* **353**, 487-498 (1974).
- Amherdt, M., Harris, V., Renold, A. E., Orci, L. & Unger, R. H. *J. clin. Invest.* **54**, 188-193 (1974).
- Mortimore, G. E. & Mondon, C. E. *J. biol. Chem.* **245**, 2375-2383 (1970).
- Neely, A. N., Cox, J. R., Fortney, J. A., Schworer, C. M. & Mortimore, G. E. *J. biol. Chem.* **252**, 6948-6954 (1977).
- Woodside, K. H. & Mortimore, G. E. *J. biol. Chem.* **247**, 6474-6481 (1972).
- Morgan, H. E. *et al. J. biol. Chem.* **246**, 2152-2162 (1971).
- Mortimore, G. E., Woodside, K. H. & Henry, J. E. *J. biol. Chem.* **247**, 2776-2784 (1972).
- Khairallah, E. A. & Mortimore, G. E. *J. biol. Chem.* **251**, 1375-1384 (1976).
- Deter, R. L. *J. Cell Biol.* **48**, 473-489 (1971).
- Kotoulas, O. B. & Phillips, M. J. *Am. J. Path.* **63**, 1-22 (1971).
- Neely, A. N., Nelson, P. B. & Mortimore, G. E. *Biochim. biophys. Acta* **338**, 458-472 (1974).
- Soberon, G. & Sanchez, Q. E. *J. biol. Chem.* **236**, 1602-1606 (1961).
- Hershko, A. & Tomkins, G. M. *J. biol. Chem.* **246**, 710-714 (1971).
- Hershko, A., Mamont, P., Shields, R. & Tomkins, G. M. *Nature new Biol.* **232**, 206-211 (1971).
- Hopgood, M. F., Clark, M. G. & Ballard, F. J. *Biochem. J.* **164**, 399-407 (1977).
- Karnovsky, M. J. *J. Cell Biol.* **27**, 137A-138A (1965).
- Loud, A. V. *J. Cell Biol.* **37**, 27-46 (1968).
- Palade, G. E. & Siekevitz, P. *J. biophys. biochem. Cytol.* **2**, 171-200 (1956).
- Rhodin, J. A. G. in *Histology* 579-606 (Oxford University Press, New York, 1974).
- Weibel, E. R., Kistler, G. S. & Scherle, W. F. *J. Cell Biol.* **30**, 23-38 (1966).
- Weibel, E. R. *Int. Rev. Cytol.* **26**, 235-302 (1969).
- Weibel, E. R., Stäubli, W., Gnägi, H. R. & Hess, F. A. *J. Cell Biol.* **42**, 68-91 (1969).
- Gilman, A. G. *Proc. natn. Acad. Sci. U.S.A.* **67**, 305-312 (1970).
- Fain, J. N., Pointer, R. H. & Ward, W. F. *J. biol. Chem.* **247**, 6866-6872 (1972).
- Steiner, A. L., Parker, C. W. & Kipnis, D. M. *J. biol. Chem.* **247**, 1106-1113 (1972).

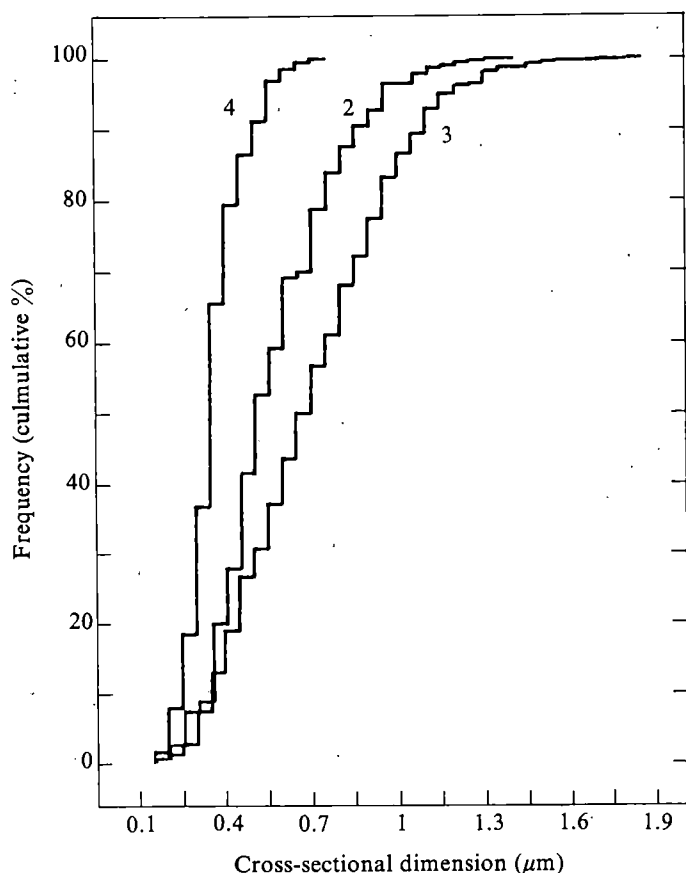


Fig. 3. Cumulative size distribution of dense bodies and autophagic elements (type A, B, and C). The data were compiled from measurements on the micrographs of Fig. 2, and the number on each curve corresponds to the experimental group as described in Fig. 2. Size estimates were obtained by directly measuring the maximum cross-sectional dimensions of profiles on electron micrographs. Dimensions were standardised by correcting for differences in magnification. Dense bodies and autophagic vacuoles were then arranged in size categories to the nearest 0.05  $\mu\text{m}$ . A total of 300 profiles were measured in group 2, 500 in group 3, and 200 in group 4.

## Monocular astigmatism effects on kitten visual cortex development

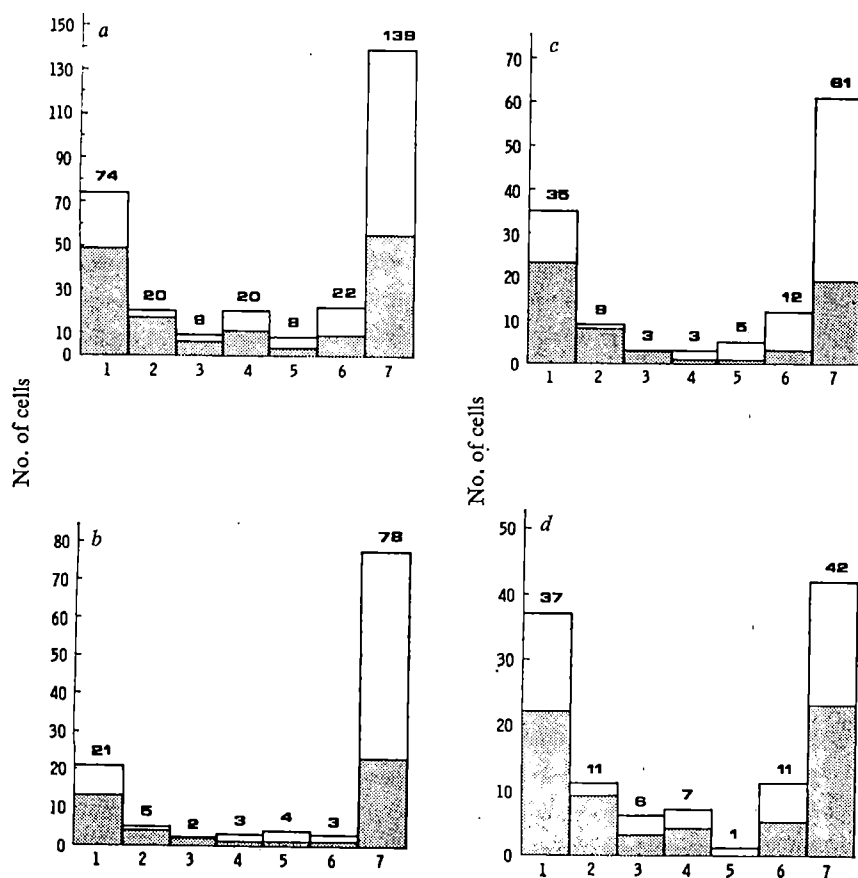
SIMULTANEOUS binocular vision during postnatal development is necessary for the maintenance of normal binocular connectivity in the kitten visual cortex. If one eyelid of a kitten is sutured shut throughout early development, three major changes can be observed when the eyelid is opened: (1) cortical cells lose functional connections with the deprived eye and may be activated only by the eye which remained open<sup>1,2</sup>; (2) cells in the layer of the lateral geniculate nucleus connected to the deprived eye shrink relative to those cells receiving innervation from the normal eye<sup>3,4</sup>; and (3) tested through its deprived eye, the cat seems to be almost totally blind<sup>5</sup>. We report here investigations on kittens which had been reared in the dark and then given visual exposure wearing goggles. One eye was allowed to view normally and the other looked through a cylindrical lens simulating astigmatism.

The behavioural, physiological and anatomical astigmatism changes which occur in the cat visual system after monocular deprivation are a result of competition between the two eyes, rather than a simple consequence of disuse<sup>6-9</sup>. Debate now centres on the mechanism of this competition. The two principle hypotheses, as put forward by Guillery<sup>6</sup> and discussed by others<sup>7-9</sup> are first, that cellular changes in the lateral geniculate body are a secondary consequence of a competitive interaction among geniculate terminals at the visual cortex. Here the primary event is the loss of functional connections with the cortical cell. Second, an intrageniculate competition mediated through inhibition between the layers of the geniculate results in a shrinkage of cells connected to the deprived eye. The loss of functional connections with the cortical units is considered to be a secondary consequence of the intrageniculate competition. We have dissociated these hypotheses by rearing kittens with

one eye viewing through a negative (axis vertical) 12-dioptre cylindrical lens whose power exceeds the accommodative range of the cat<sup>10</sup>. This lens allows clear vision of horizontal contours but defocuses the image progressively more as the stimulus orientation approaches vertical. As such it simulates the clinical condition of astigmatism<sup>11</sup>.

Three kittens were reared from birth to 25 d old in the dark. They were then given visual exposure wearing goggles which forced one eye to view through the cylindrical lens while the other viewed normally, for 4-6 h per d for a total of 80-120 h. They were then returned to the dark until they were at least 3-months-old at which point single cell responses were examined in visual cortex. Our procedures for recording responses from single cortical cells are described elsewhere<sup>12,13</sup>. Kittens were initially anaesthetised with intravenous sodium thiopental and cortical units were recorded extracellularly with glass-coated platinum-iridium micro-electrodes. We used the sampling methods of Stryker and Sherk<sup>17</sup>, sampling units at intervals of approximately 100  $\mu$ m in order to minimise the bias of our recorded population. A blind procedure in which the experimenter knew neither the axis of the cylindrical lens, nor which eye wore the negative lens was used throughout the recording sessions. If the competition between the two eyes occurs at the orientation-selective cortical cell, the effect of the lens should be to give the normal eye a competitive advantage at cells preferring vertical stimuli and no competitive advantage at cells preferring horizontal stimuli. The 'cortical' mechanism proposed by Guillery<sup>6</sup>, thus predicts that the effect of the deprivation would be maximal at vertically-orientated cells and minimal at horizontally-orientated cells. If the competition occurs at a more peripheral level, where orientation selectivity among the cells is not present, the 'geniculate' mechanism predicts that the deprivation should reduce the effectiveness of the 'astigmatic' eye equally across cortical units of all orientations.

The distribution of ocular dominance for 292 units encountered in these kittens is shown in Fig. 2. As can be



**Fig. 1** The distribution of ocular dominance for units in the visual cortex of cats reared with one eye viewing through a cylindrical lens and the other eye viewing normally. The numbers on the abscissa from 1 to 7 represent a trend from the 'astigmatic' eye to the normal eye. Cells in group 1 are driven exclusively by the astigmatic eye; cells in group 4 are driven equally by both eyes; cells in group 7 exclusively by the normal eye. *a*, The distribution of ocular dominance for all cells in these cats. *b*, *c* and *d*, the distribution of ocular dominance as a function of the orientation of the unit. *b*, The vertical and *d*, the horizontal distributions represent cells which prefer orientations within 30° of vertical and horizontal. *c*, The 'diagonal' distribution represents cells preferring stimulus orientations within 15° of either diagonal. The hatched parts of the distribution represent cells in the hemisphere contralateral to the astigmatic eye.

seen, about twice as many units can be influenced through the normal eye as through the astigmatic eye. The distribution of ocular dominance is broken down according to the preferred orientation of the cortical units in the lower parts of Fig. 1. It is clear that the magnitude of the deprivation effect varies depending on the orientation preference of the cortical cell. It is marked for units preferring orientations within  $30^\circ$  of vertical, weaker for diagonally-orientated units and absent for units preferring horizontally-orientated ( $\pm 30^\circ$ ) stimuli. The results thus confirm the cortical hypothesis outlined earlier; they show that binocular competition occurs at the orientation-selective cortical cell and that this competition seems to be sufficient to account for all ocular dominance changes consequent to monocular image blur.

The total number of units encountered which prefer a particular orientation is shown for each eye separately in Fig. 2. The 'astigmatic' eye drives far fewer units which prefer vertically-orientated stimuli than units which prefer horizontal. The histograms in Fig. 2 show that the effect is similar in both hemispheres. One might imagine that this is a simple consequence of this eye having less exposure to focused stimuli with vertical orientations<sup>14-17</sup>. Such an explanation cannot, however, account for the striking bias in the distribution of preferred orientations which is evident in the normally-viewing eye. In this eye, 55% more cells prefer stimuli orientated within  $30^\circ$  of vertical than prefer stimuli within  $30^\circ$  of horizontal. This is at first glance surprising, since the normal eye has, after all, viewed all orientations with equal frequency and clarity. An examination of the histograms of Fig. 3 reveals that the distributions in the two eyes are complementary. The trough near vertical for the astigmatic eye is balanced by a corresponding bulge in the normal eye. Combining distributions for the two eyes, the total number of units encountered preferring vertical ( $\pm 30^\circ$ ) and horizontal ( $\pm 30^\circ$ ) is nearly identical. The overall distribution of orientation-selective

neurons in the visual cortex has remained unaltered but the normal eye seems to have made compensatory, orientation-selective inroads into the cortical territory normally occupied by the astigmatic eye.

The effects obtained here are not as marked as those which follow monocular eyelid suture during early development<sup>1,2</sup>. The latter case differs from ours in that the sutured eyelid almost completely obscures the stimulus while our negative lens merely blurs the image. The observed effects seem more marked in the hemisphere contralateral to the eye which wore the negative lens. This apparent asymmetry is probably attributable to the known preponderance of input from the contralateral eye to the visual cortex<sup>18</sup>. The normal contralateral bias would enhance the observed effects in one hemisphere and minimise them in the other.

A puzzle remains in the ocular dominance distribution for horizontally-orientated units where viewing conditions for the two eyes should be equal. While there is no tendency for either eye to control more neurones, the distribution lacks the usual complement of binocular neurones. The most likely explanation for this phenomenon is that the cylindrical lens was not perfectly centred on the kitten's eye. Even a small misalignment between the geometrical centre of the lens and the pupil would lead to prismatic displacement which could vary from day to day. This would mimic the situation in strabismus, a condition which is known to result in a loss of binocularly-driven neurones<sup>19</sup>.

Three main conclusions may be drawn from these results: first, asymmetric ocular refractive error during early development can cause severe changes in cortical binocular connectivity. Second, the mechanism of this disruption is a developmental competition between the two eyes which occurs at the visual cortex. And third, the cortical distribution of preferred orientations in a normally-viewing eye can be biased merely by depriving the other eye of focused input at certain orientations.

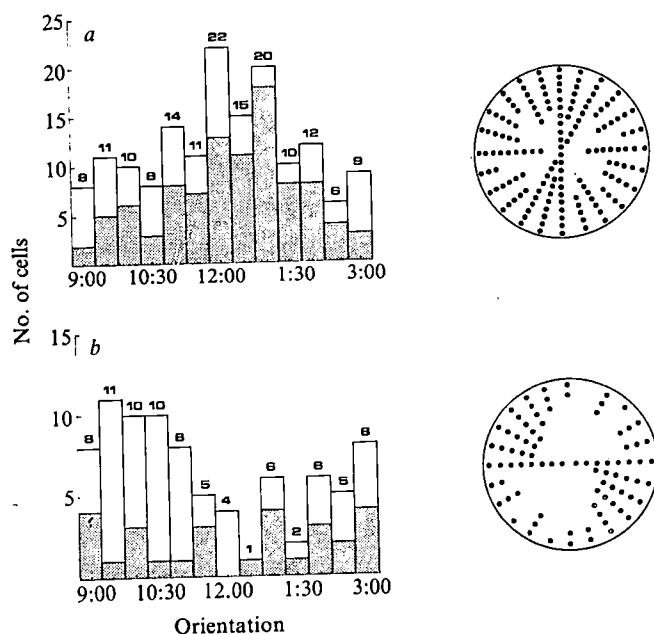
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1. Wiesel, T. N. & Hubel, D. H. *J. Neurophysiol.* **26**, 1003-1017 (1963).
2. Hubel, D. H. & Wiesel, T. N. *J. Neurophysiol.* **28**, 1029-1040 (1965).
3. Wiesel, T. N. & Hubel, D. H. *J. Neurophysiol.* **26**, 978-993 (1963).
4. Kupfer, C. & Palmer, P. *Expl. Neurol.* **9**, 400-409 (1964).
5. Dews, P. B. & Wiesel, T. N. *J. Physiol., Lond.* **206**, 437-455 (1970).
6. Guillery, R. W. *J. comp. Neurol.* **144**, 117-127 (1972).
7. Guillery, R. W. *J. comp. Neurol.* **148**, 417-422 (1973).
8. Sherman, S. M., Guillery, R. W., Kaas, J. H. & Sanderson, K. S. *J. comp. Neurol.* **148**, 1-18 (1976).
9. Cragg, B. G., Anker, R. & Wan, Y. K. *J. comp. Neurol.* **168**, 345-357 (1976).
10. Bloom, M. & Berkley, M. A. *Vis. Res.* **17**, 723-730 (1977).
11. Mitchell, D. E., Freeman, R. D., Millodot, M. & Haegerstrom, G. *Vis. Res.* **13**, 535-558 (1973).
12. Cynader, M., Berman, N. & Hein, A. *Expl. Brain Res.* **25**, 139-156 (1976).
13. Cynader, M. & Berman, N. *J. Neurophysiol.* **35**, 187-201 (1972).
14. Freeman, R. D. & Pettigrew, J. D. *Nature* **246**, 359-361 (1973).
15. Blakemore, C. & Cooper, C. *Nature* **228**, 477-478 (1970).
16. Hirsch, H. V. B. & Spinelli, N. *Science* **168**, 869-871 (1970).
17. Stryker, M. P. & Sherk, H. *Science* **190**, 904-905 (1975).
18. Hubel, D. H. & Wiesel, T. N. *J. Physiol., Lond.* **160**, 106-154 (1962).
19. Hubel, D. H. & Wiesel, T. N. *J. Neurophysiol.* **28**, 1041-1059 (1965).



**Fig. 2** Distribution of preferred orientations for cortical units driven by *a*, the normal and *b*, the astigmatic eyes. On the right are polar plots in which each cell represents a single dot. The length of the dotted line along a given orientation is thus proportional to the number of units encountered which have this preferred orientation. On the left, the same data are presented in histogram form and are further subdivided by cortical hemisphere. The height of the bar at any orientation is proportional to the number of units preferring that orientation. The hatched part of the distribution is derived from the hemisphere contralateral to the astigmatic eye. The horizontal cells of the polar plots are divided equally between 9:00 and 3:00 on the associated histograms.

## Diffusion barrier for acetylcholine at the frog neuromuscular junction

INACTIVATION of acetylcholinesterase (AChE) causes the half-decay times of the endplate currents (e.p.cs) and miniature e.p.cs (m.e.p.cs) to increase from about 1.5-2.0 ms to 3.0-10.00 ms, times far too long to be accounted for by removal of the transmitter by free diffusion<sup>1-3</sup>. To explain this, Katz and Miledi<sup>1</sup> postulated

leptics inhibit  $^3\text{H}$ -haloperidol and  $^3\text{H}$ -spiroperidol binding in concentrations substantially lower than those which occur in the blood of patients at therapeutic doses. The radioreceptor assay described here is based on the principle that neuroleptics in the patient's serum compete for the binding of  $^3\text{H}$ -haloperidol or  $^3\text{H}$ -spiroperidol to dopamine receptors in membranes of the corpus striatum (S. H. S., unpublished).

In this assay, serum is used without any extraction or other purification procedure. The absence of interference by serum is apparent from experiments in which 15  $\mu\text{l}$  of serum in a total assay volume of 1 ml reduced  $^3\text{H}$ -haloperidol or  $^3\text{H}$ -spiroperidol binding by only 10–15%. Increasing volumes of serum reduced binding linearly with about 30% inhibition occurring with 150  $\mu\text{l}$  of serum. Small volumes of serum reduce both specific and non-specific  $^3\text{H}$ -ligand binding to similar extents suggesting that serum proteins bind  $^3\text{H}$ -ligand, making less available for interactions with brain membranes. The degree of inhibition of  $^3\text{H}$ -ligand binding by control sera from several laboratory personnel was uniform. Specific binding of  $^3\text{H}$ -haloperidol in the presence of 15  $\mu\text{l}$  of serum from 18 different subjects was  $91 \pm 1.6\%$  (total binding 2,325 c.p.m. blank with 0.1 mM dopamine 953 c.p.m.) of control values while specific  $^3\text{H}$ -spiroperidol binding was  $85 \pm 1.2\%$  (total binding 1,264 c.p.m. blank with 1 mM dopamine 191 c.p.m.) of control for 10 different subjects. This small degree of variability in inhibition of  $^3\text{H}$ -ligand binding by control sera falls within the error for repeated determinations of the same sample.

Neuroleptic present in a serum sample reduces  $^3\text{H}$ -ligand binding beyond the small reduction attributable to serum alone and the degree of inhibition of specific  $^3\text{H}$ -ligand binding is proportional to the amount of neuroleptic present. By constructing a standard curve of inhibition of specific  $^3\text{H}$ -ligand binding by known amounts of the drug, the amount present in a serum sample can be easily determined.

This method requires that the presence or absence of neuroleptic in a serum sample will not affect the degree of nonspecific (blank)  $^3\text{H}$ -ligand binding. In 46 serum samples from patients on neuroleptics, blank  $^3\text{H}$ -haloperidol binding was  $887 \pm 6$  c.p.m. while nonspecific binding in the presence of five control sera was  $894 \pm 17$  c.p.m. In six patients on haloperidol, nonspecific  $^3\text{H}$ -spiroperidol binding was  $199 \pm 4$  c.p.m. and  $198 \pm 6$  c.p.m. in the presence of six control serum samples. Thus specific binding in the presence of patient sera can be determined by subtracting nonspecific binding values obtained with control sera from total binding with patient sera. The percentage inhibition of specific  $^3\text{H}$ -ligand binding (in the presence of control serum) by the patient serum (containing drug) is then calculated and compared to a standard displacement curve for determining actual neuroleptic content.

Up to 99% of neuroleptics may be bound to blood components<sup>23,24</sup>. In this assay the neuroleptic bound to blood proteins dissociates during the incubation so that total neuroleptic levels are measured. In confirmation of this assumption in one experiment 15 nM haloperidol was preincubated for 10 min at 37 °C with control serum to allow binding to serum proteins to occur. The serum and haloperidol was then added to a standard binding assay which was at equilibrium and it progressively reduced  $^3\text{H}$ -haloperidol binding with increasing durations of incubation, with a maximal lowering by 5–10 min. The time course and extent of reduction of binding was the same for haloperidol pre-incubated with serum or simply dissolved in water. The maximum percentage lowering of binding was equivalent to that produced by 15 nM haloperidol added directly to the assay with no serum present.

For the neuroleptic radioreceptor assay fresh rat striatum was sonicated in 100 volumes (w/v) 50 mM Tris buffer, pH 7.7 at 25 °C with a Sonifier, setting 4 for 30 s. The homogenate was centrifuged twice at 50,000g for 10 min (Sorvall RC2-B) with rehomogenisation of the intermediate pellet in fresh buffer. The final pellet (which may be stored frozen) was resuspended in 125 volumes, for assays using  $^3\text{H}$ -haloperidol, or 285 volumes for  $^3\text{H}$ -spiroperidol, of freshly prepared 50 mM Tris buffer containing 0.1% ascorbic acid, 10  $\mu\text{M}$  pargyline, 120 mM NaCl, 5 mM KCl, 2 mM  $\text{CaCl}_2$  and 1 mM  $\text{MgCl}_2$  to give a final pH of 7.1 at 37 °C.  $^3\text{H}$ -Haloperidol (15 Ci mmol<sup>-1</sup>, New England Nuclear) or  $^3\text{H}$ -spiroperidol (26 Ci mmol<sup>-1</sup>, New England or Amersham) was diluted to 10 nM or 1 nM, respectively, in fresh 0.1% ascorbic acid. Glass 12  $\times$  75 mm incubation tubes received, in order, 15–150  $\mu\text{l}$  serum (on drug therapy or drug free), 100  $\mu\text{l}$   $^3\text{H}$ -ligand, 100  $\mu\text{l}$  drug for standard curve or dopamine for blanks ( $10^{-4}$  M for  $^3\text{H}$ -haloperidol and  $10^{-3}$  M for  $^3\text{H}$ -spiroperidol) or the drug solvent 0.1% ascorbic acid, and tissue suspension to 1 ml total volume. Final concentrations of  $^3\text{H}$ -haloperidol or  $^3\text{H}$ -spiroperidol were 1 nM and 0.1 nM respectively. The tubes were incubated at 37 °C for 10 min ( $^3\text{H}$ -haloperidol) or 15 min ( $^3\text{H}$ -spiroperidol) and rapidly filtered under vacuum through Whatman GF/B filters with three 5-ml rinses of ice-cold 50 mM Tris buffer, pH 7.7 at 25 °C.  $^3\text{H}$ -Ligand trapped on the filters was counted by liquid scintillation spectrometry after remaining overnight in scintillation vials containing NEN formula 947 fluor (New England Nuclear). A standard displacement curve for the drug under study was determined in the presence of equal volumes of control serum with final concentrations of the drug about one-third, three times and the same as its  $K_i$  value determined previously<sup>19,22</sup>. A log-probit plot was used to convert the displacement curve to a straight line so percentage inhibition of  $^3\text{H}$ -ligand binding can be easily converted to molar drug concentration (Fig. 1). In the presence of serum the ratio of total to nonspecific binding was about 2.4 for  $^3\text{H}$ -haloperidol and 6.6 for  $^3\text{H}$ -spiroperidol, so that the latter is the preferred ligand. Plasma can be used for assays if the KCl,  $\text{CaCl}_2$  and  $\text{MgCl}_2$  in the buffer are replaced by 5 mM sodium EDTA which prevents the plasma from clotting the brain homogenate. Neuroleptics can also be eluted from red blood cells by sonification and incubation in 0.1% ascorbic acid.

To examine recovery five neuroleptics were preincubated with serum for 10 min at 37 °C, to provide time for binding to serum proteins and subsequently assayed for

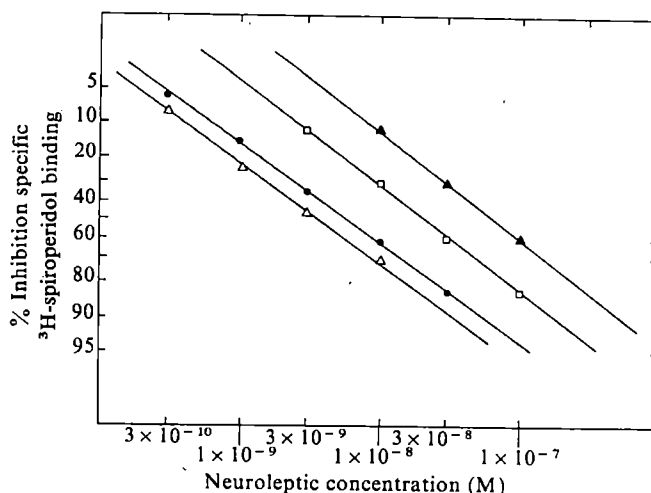


Fig. 1 Log-probit plots of neuroleptic inhibition of specific  $^3\text{H}$ -spiroperidol binding to rat striatal membranes.  $\blacktriangle$ , Thiordiazine;  $\square$ , chlorpromazine;  $\bullet$ , haloperidol;  $\triangle$ , fluphenazine.



neuroleptic levels. Haloperidol (0.1  $\mu$ M), fluphenazine (0.1  $\mu$ M), trifluoperazine (0.1  $\mu$ M), chlorpromazine (1  $\mu$ M), and thioridazine (1  $\mu$ M) were all fully recovered in the neuroleptic radioreceptor assay with respective values of  $0.11 \pm 0.02 \mu$ M ( $n=6$ ),  $0.12 \pm 0.01 \mu$ M ( $n=10$ ),  $0.16 \pm 0.2 \mu$ M ( $n=6$ ),  $1.0 \pm 0.1 \mu$ M ( $n=9$ ) and  $1.1 \pm 0.2 \mu$ M ( $n=6$ ). To examine the recovery of neuroleptics from the blood of patients treated *in vivo* we measured blood levels in four patients receiving oral doses of haloperidol both after extraction into organic solvent (heptane-5% isoamyl-alcohol, back extracted into 0.1% ascorbic acid) which should remove all drug bound to serum protein, and by adding serum directly to the binding assay as in the above method. Serum drug levels ranged between 10 and 250 nM and agreed within a mean of 10% whether assays were conducted with or without extraction.

To assess the validity of the radioreceptor assay, we compared its results with blood levels measured by other procedures. In one study, rats were given  $^3$ H-haloperidol 0.2 mg per kg body weight and blood levels of the drug were assessed by determining plasma radioactivity 10 min after drug administration or by radioreceptor assay following organic solvent extraction of the plasma. At this interval after  $^3$ H-haloperidol administration at least 75% of the radioactivity in plasma can be accounted for as unmetabolised haloperidol<sup>23</sup>. The blood level was  $130 \pm 30$  nM by measuring radioactivity and  $120 \pm 30$  nM by radioreceptor assay. In other studies, we measured blood haloperidol levels in patients whose levels were also determined by radioimmunoassay and found a good agreement between the two procedures (Table 1) (I.C., R. J. Wyatt and S.H.S., in preparation). Serum samples have been assayed fresh or after storage at or below  $-20^\circ\text{C}$ , but repeated freezing and thawing led to reduced measured neuroleptic levels. Using 150  $\mu$ l of serum the lower limit of sensitivity (15% inhibition of specific binding) is 1.8 ng ml<sup>-1</sup>, 2.2 ng ml<sup>-1</sup>, 2.5 ng ml<sup>-1</sup>, 8.6 ng ml<sup>-1</sup> and 30 ng ml<sup>-1</sup> for fluphenazine, trifluoperazine, haloperidol, chlorpromazine and thioridazine, respectively.

**Table 1** Comparison of radioreceptor assay (RRA) and radioimmunoassay (RIA) values for plasma levels of haloperidol

Patient samples	Dose level, Haldol (mg d <sup>-1</sup> )	Haloperidol (ng ml <sup>-1</sup> )	
		RRA	RIA
1	20	27	27
2	20	29	28
3	10	21	18
4	20	31	37
5	10	22	20
6	20	29	28

Aliquots of 30  $\mu$ l of patient plasma samples (previously frozen at  $-20^\circ\text{C}$ ) were assayed in the radioreceptor assay with  $^3$ H-haloperidol as ligand. A standard curve of  $^3$ H-haloperidol displacement was constructed from known amounts of haloperidol in the presence of control plasma. The radioimmunoassay<sup>28</sup> for haloperidol was performed by Drs Michael Shostak and James Perel, Columbia University.

In summary, this radioreceptor assay seems to offer a potential for routine application to large populations of patients. The procedure is sensitive. Since the milligram potencies of neuroleptics are closely correlated with their affinity for dopamine receptors labelled with  $^3$ H-haloperidol<sup>19,21</sup>, the sensitivity of the assay is greater for drugs which are used therapeutically at lower doses. At therapeutic doses of many neuroleptics, we have found the radioreceptor assay sensitive enough to determine drug levels in 15–30  $\mu$ l serum samples. But, where steady state blood levels are relatively low the sensitivity of the assay can be increased up to 10-fold by increasing the volume of serum (up to 150  $\mu$ l) added to the assay. The assay is selective for neuroleptics. Only dopamine receptor

antagonists compete with high affinity for  $^3$ H-haloperidol and  $^3$ H-spiroperidol binding. Levels of circulating catecholamines are much too low to pose any problem. In screens of large numbers of drugs from different chemical classes no drugs other than neuroleptics, except for ergots, compete significantly for  $^3$ H-haloperidol binding<sup>26</sup>. Ergots which do bind to dopamine receptors are not usually used clinically in the therapy of schizophrenics. Thus, this assay can be used in patients who are also receiving a variety of other drugs besides neuroleptics. For patients treated with two or more different neuroleptics, the absolute concentration of each drug cannot be separately determined. In these cases the total inhibition of  $^3$ H-haloperidol or  $^3$ H-spiroperidol binding can be converted to a 'chlorpromazine equivalent' which would produce the same degree of inhibition.

Another virtue of the assay is that it will detect metabolites which compete for dopamine receptor binding and thus may be therapeutically active. There is evidence that a substantial portion of the therapeutic activity of chlorpromazine might be contributed by its metabolite 7-hydroxychlorpromazine<sup>27</sup>. 7-Hydroxychlorpromazine has about 70% of the affinity of chlorpromazine itself for  $^3$ H-haloperidol binding sites<sup>28</sup> and hence would be detected in this assay.

The radioreceptor assay described here is simple to perform. Substantial quantities of corpus striatum tissue can be obtained from large animals such as calf or pig and stored frozen.  $^3$ H-Haloperidol and  $^3$ H-spiroperidol are commercially available. As many as 100 assays can be conducted in a morning.

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- Curry, S. H., Davis, J. M. & Janowsky, D. S. *Archs gen. Psychiat.* 22, 209–215 (1970).
- Garver, D. L. *et al. Archs gen. Psychiat.* 33, 862–866 (1976).
- Cressman, W. A., Bianchine, J. R., Slotnick, V. B., Johnson, P. C. & Plostneiks, S. J. *Eur. J. clin. Pharmac.* 7, 99–103 (1974).
- Forsman, A., Martensson, E., Nyberg, G. & Ohman, R. *Naunyn-Schmiedeberg's Arch. Pharmac.* 286, 113–124 (1974).
- Kobayashi, R. M. *New Engl. J. Med.* 296, 257–260 (1977).
- American College of Neuropsychopharmacology—F.D.A. Task Force, *New Eng. J. Med.* 289, 20–23 (1973).
- Curry, S. H., Marshall, J. H. L., Davis, J. M. & Janowsky, D. S. *Archs gen. Psychiat.* 22, 289–296 (1970).
- Rivera-Calimlim, L., Nasrallah, H., Straus, J. & Lasagna, L., *Am. J. Psychiat.* 133, 646–652 (1976).
- Garver, D. L., Dekirmenjian, H., Davis, J. M., Cooper, R. & Erickson, S. *Am. J. Psychiat.* 134, 304–307 (1977).
- Usdin, E., *CRC Crit. Rev. clin. Lab. Sci.* 2, 347–391 (1971).
- Marcucci, F., Airolidi, L., Mussini, E. & Garattini, S. *J. Chromatogr.* 59, 174–177 (1971).
- Zingales, I. A. *J. Chromatogr.* 54, 15–24 (1971).
- Alfredsson, G., Wode-Helgott, B. & Sedvall, G. *Psychopharmacologia* 48, 123–131 (1976).
- Rivera-Calimlim, L. & Siracusa, A. *Comm. Psychopharmac.* 1, 233–242 (1977).
- Kawashima, K., Dixon, R. & Spector, S. *Eur. J. Pharmac.* 32, 195–202 (1975).
- Clark, B. R., Tower, B. B. & Rubin, R. T. *Life Sci.* 20, 319–326 (1977).
- Snyder, S. H., Banerjee, S. P., Yamamura, H. I. & Greenberg, D. A. *Science* 184, 1243–1253 (1974).
- Iversen, L. L. *Science* 188, 1084–1089 (1975).
- Cree, I., Burt, D. R. & Snyder, S. H. *Science* 194, 481–483 (1976).
- Cree, I., Burt, D. R. & Snyder, S. H. *Science* 194, 546 (1976).
- Seeman, P., Lee, T., Chau-Wong, M. & Wong, K. *Nature* 261, 717–719 (1976).
- Cree, I., Schneider, R. & Snyder, S. H. *Eur. J. Pharmac.* (in the press).
- Curry, S. H. *J. Pharm. Pharmac.* 22, 193–197 (1969).
- Bickel, M. H. *J. Pharm. Pharmac.* 27, 733–738 (1975).
- Janssen, P. A. J. & Allewijn, F. T. N. *Arzneim. Forsch.* 19, 199–208 (1969).
- Burt, D. R., Cree, I. & Snyder, S. H. *Molec. Pharmac.* 12, 800–812 (1976).
- Sakalis, G., Chan, T. L., Gershon, S. & Park, S. *Psychopharmacologia* 32, 279–284 (1973).
- Cree, I., Manian, A. A., Prosser, T. D. & Snyder, S. H. *Eur. J. Pharmac.* (in the press).
- Shostak, M. & Perel, J. M. *Fedn Proc.* 35, 531 (1976).



## Specific localisation of neurotensin to the N cell in human intestine by radioimmunoassay and immunocytochemistry

NEUROTENSIN, originally isolated from bovine hypothalamus<sup>1</sup>, was shown to be a peptide of 13 amino acids<sup>2</sup>. It has been synthesised<sup>3</sup> and its pharmacological actions shown to include effects on small intestine motility, blood vessel tone, enhancement of glycogenolysis, release of glucagon and inhibition of insulin release<sup>4-7</sup>. Immunoreactive neurotensin has been detected in mammalian hypothalamus and a few other areas of the brain<sup>8-10</sup> but much larger quantities occur in the intestine<sup>11</sup>. Neurotensin has been shown in discrete cells in the ileal mucosa of various birds and mammals including man<sup>12-15</sup>. The cell type has recently been fully characterised and its identity internationally accepted (see ref. 16). The distribution of the peptide in man is unknown. Using a combination of radioimmunoassay (RIA) and immunocytochemistry (IC) we have found large amounts of neurotensin-like reactivity in human ileum with less in the jejunum. The immunoreactive cells are restricted to the mucosa. Neurotensin thus joins the growing group of peptides which are common to brain and gut<sup>17</sup>. The effect of such peptides may differ according to their location. Neurotensin has been reported in rat blood<sup>11</sup> and may therefore act as a circulating hormone. In addition, its location in the brain suggests a possible role as a peptidergic neurotransmitter.

Fresh samples of human gastrointestinal tract were obtained during surgery for various disease states and were later found to be histologically normal. These included body and antrum of stomach, duodenum, jejunum, ileum, colon, pancreas and gall bladder. Part of the material was fixed in *p*-benzoquinone vapour<sup>18</sup> and part in purified glutaraldehyde<sup>19</sup> for ultrastructural studies. Pairs of serial 1- $\mu$ m resin-embedded sections were treated by an indirect immunocytochemical procedure with antisera to neurotensin and to one of the following peptides: somatostatin, glucagon, vasoactive intestinal peptide (VIP), gastrin, gastric inhibitory peptide (GIP) and cholecystokinin (CCK). The controls used included previous absorption of the antisera with glucagon, somatostatin, VIP, CCK, gastrin, GIP and neurotensin, at concentrations ranging from 2 to 25  $\mu$ g per ml of diluted antisera. Negative staining with anti-neurotensin was obtained only after inactivation with at least 10  $\mu$ g of synthetic neurotensin per ml of diluted antibody. Negative staining was also obtained by the use of normal rabbit serum instead of immune serum.

For radioimmunoassay a part of each surgical sample was extracted by boiling for 5 min in distilled water and two homogenisations. The tissue was then further extracted in 0.1 M formic acid. Both water and acid extracts were assayed at 10-fold serial dilutions. Antibodies for immunocytochemistry and radioimmunoassay were raised in rabbits to synthetic neurotensin coupled by carbodiimide to bovine serum albumin and by glutaraldehyde to bovine thyroglobulin. <sup>125</sup>I-Neurotensin was prepared by chloramine T oxidation and was stable at -20 °C for several months. The assay was sensitive to 10 pmol per tube and showed no cross reaction with any other gut hormone. Antibody interference by nonspecific effects was negligible, but was anyway unlikely because the assay sensitivity necessitated assay of extracts only in considerable dilution.

Many endocrine cells, stained solely with antibodies to neurotensin, were located in a restricted area of the gut, being especially numerous in the ileal mucosa. Their number diminished as the jejunum was reached and they were extremely sparse in the duodenum. Neurotensin immunoreactive cells were not detected in the mucosa of

stomach, pancreas or colon, or in any structure in the other layers of the gut wall. They were situated in the upper two-thirds of the villi (Fig. 1a) and were often seen to communicate with the intestinal lumen by means of microvilli (Fig. 1b). The cells were oval in shape and their secretory granules, located mainly in the basal parts of the cell (Fig. 1b), had an average size of 300 nm and a closely attached membrane (Fig. 1c). The neurotensin immunoreactive cells were not members of the argentaffin subgroup of APUD cells<sup>20</sup> since they were unstained with the Masson silver impregnation technique.

When serial 1- $\mu$ m sections were stained with antibodies reacting with enteroglucagon (EG) and VIP the positive cells were completely distinct from those stained by the neurotensin antibodies. The EG-positive cells contained

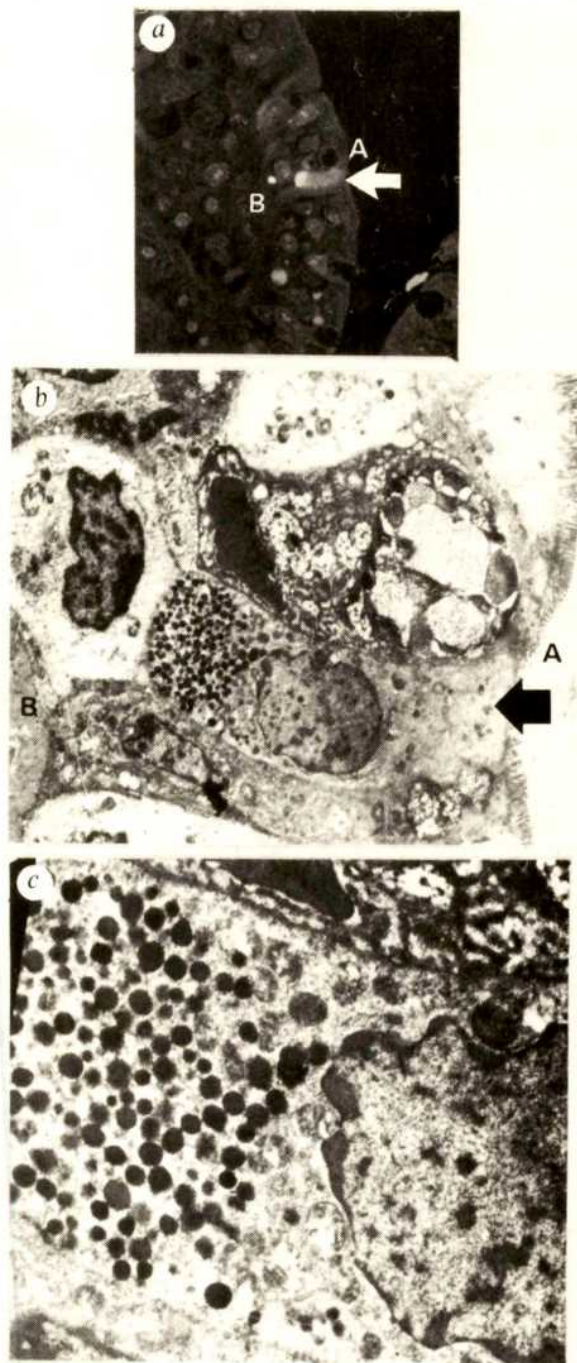


Fig. 1a, 1- $\mu$ m Araldite section stained for neurotensin. Arrow marks positive cell. A, gut lumen; B, lamina propria.  $\times 625$ . b, Serial 40-nm section showing same cell at EM. Arrow marks cell. A, gut lumen; B, lamina propria.  $\times 4,000$ . c, High magnification of cell to show detail of secretory granules.  $\times 14,000$ .



round granules with a mean diameter of approximately 260 nm and a closely attached membrane, which thus were considerably smaller than those of the N cell. The VIP-positive cells varied in shape and contained much smaller granules with a mean diameter of 120 nm and a closely attached membrane.

Thus neurotensin is confined to a single type of endocrine cell which has previously been overlooked in ultrastructural studies because of confusion with the EG(L) cells<sup>21</sup>. With the application of electron immunocytochemical methods it can now be seen that the cell contains granules of a type distinct from those of the other endocrine cells in the ileal mucosa.

Neurotensin immunoreactivity was detected by radioimmunoassay in high concentration in the ileal mucosa with lesser quantities in the jejunal mucosa (Table 1). Very much smaller quantities were found in whole thickness extracts of the wall of the stomach, duodenum and colon. It was undetectable in the pancreas and gall bladder.

**Table 1** Neurotensin immunoreactivity in various tissues

	Neurotensin (pmol per g wet weight)
Stomach (6)* full thickness	0.27 ± 0.03
Duodenum (7) full thickness	0.24 ± 0.10
Jejunum full thickness (2) mucosa (3)	0.80 (1.19, 0.42) 2.82 (4.62, 1.78, 2.07)
Ileum full thickness (2) mucosa (3)	12.0 (7.57, 16.4) 16.2 (23.1, 19.0, 6.5)
Colon (4)	0.50 ± 0.19
Gall bladder (3)	< 0.03
Pancreas (2)	< 0.03

Values are means ± s.e.m.

\*No. of determinations.

Neurotensin was originally extracted from the hypothalamus<sup>1</sup>, and its function there is no more clear than in the gastrointestinal tract. The morphological features of the neurotensin cells, which have microvilli and secretory granules located at the vascular pole, suggest they are sensitive to changes in the gut lumen which may thus provide the stimulus for the release of the peptide, presumably, because of the basal distribution of the neurotensin granule, directly into the circulation. This view is supported by the finding of neurotensin in rat plasma<sup>11</sup>. The restricted localisation of the peptide suggests that it may play a precise part in the sequence of post-digestive processes.

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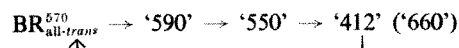
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1. Carraway, R. & Leeman, S. E. *J. biol. Chem.* **248**, 6854–6861 (1973).
2. Carraway, R. & Leeman, S. E. *J. biol. Chem.* **250**, 1907–1911 (1975).
3. Carraway, R. & Leeman, S. E. *J. biol. Chem.* **250**, 1912–1918 (1975).
4. Brown, M., Villarreal, J. & Vale, W. *Metabolism* **25**, 1459–1461 (1976).
5. Nagai, K. & Frohman, L. A. *Life Sci.* **19**, 273–280 (1976).
6. Carraway, R., Demers, L. & Leeman, S. E. *Endocrinology* **99**, 1452–1462 (1976).
7. Brown, M. & Vale, W. *Endocrinology* **98**, 819–822 (1976).
8. Carraway, R. & Leeman, S. E. *J. biol. Chem.* **251**, 7035–7044 (1976).
9. Uhl, G. R. & Snyder, S. H. *Life Sci.* **19**, 1827–1832 (1976).

10. Kobayashi, R. M., Brown, M. & Vale, W. *Brain Res.* (in the press).
11. Carraway, R. & Leeman, S. E. *J. biol. Chem.* **251**, 7045–7052 (1976).
12. Orci, L. *et al. Life Sci.* **19**, 559–562 (1976).
13. Sundler, F., Alumets, J., Håkanson, R., Carraway, R. & Leeman, S. E. *Histochemistry* **53**, 25–34 (1977).
14. Helmstaedter, V., Taugner, C., Feurle, G. E. & Forssman, W. G. *Histochemistry* **53**, 35–41 (1977).
15. Buchan, A. M. J. *et al.* in *Gut Hormones* (ed. Bloom, S. R.) (Churchill-Livingstone, London, in the press).
16. Bloom, S. R. (ed.) *Gut Hormones* (Churchill-Livingstone, London, in the press).
17. Pearse, A. G. E. *Nature* **262**, 92–94 (1976).
18. Pearse, A. G. E. & Polak, J. M. *Histochemistry* **27**, 96–102 (1971).
19. Polak, J. M., Sullivan, S. N., Bloom, S. R., Facer, P. & Pearse, A. G. E. *Lancet* **i**, 972–974 (1977).
20. Pearse, A. G. E., Polak, J. M. & Bloom, S. R. *Gastroenterology* **72**, 746–761 (1977).
21. Solcia, E., Capella, C., Vassallo, G. & Buffa, R. *Int. Rev. Cytol.* **45**, 223–286 (1973).

## Hydration effects on the photocycle of bacteriorhodopsin in thin layers of purple membrane

THE purple membrane of *Halobacterium halobium* acts as a light-driven proton pump, producing a transmembrane proton gradient which is coupled to ATP synthesis<sup>1</sup>, and to phototaxis<sup>2</sup> in the intact bacteria. It contains a single type of protein, bacteriorhodopsin (BR) which spans a 45-Å membrane. The isolated purple membranes are flat oval sheets with an average diameter of 0.5 µm (refs 3, 4). Bacteriorhodopsin contains a retinal molecule (all-*trans* and 13-*cis*)<sup>5</sup> which is covalently bound via a protonated Schiff base to a lysine residue. It undergoes a photocycle described by the following scheme<sup>6–8</sup>:



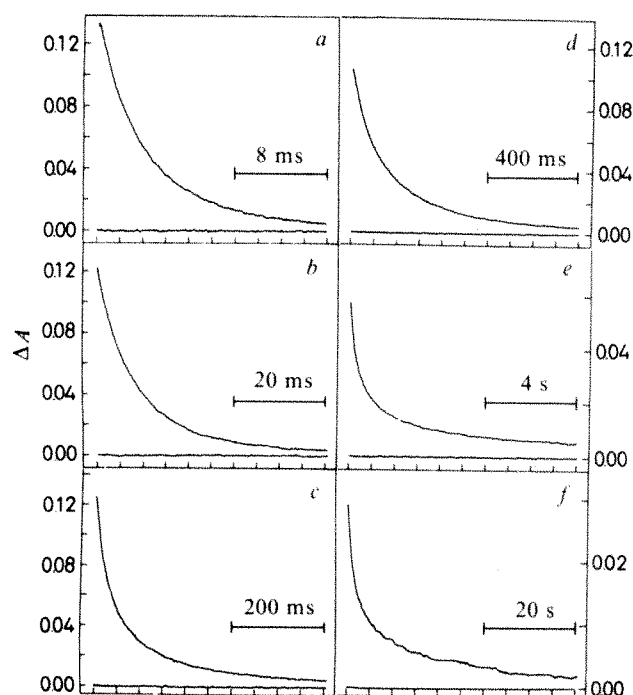
where proton ejection to the bulk solution occurs on the route '550' → '412' (refs 9, 10), whereas protonation of the bacteriorhodopsin takes place parallel to the '412' → BR<sup>570</sup><sub>all-trans</sub> process<sup>11</sup>. It has been reported that the reconstituted BR<sup>548</sup><sub>13-cis</sub> undergoes a cycle which involves the 'X' and the '610' intermediates<sup>12</sup>. It was demonstrated that proton transfer is a vectorial process where the proton is ejected from one side of the purple membrane and reprotonation takes place on the other side<sup>13</sup>. We present here results on the effects of the specific hydration of the purple membrane on the relaxation times of '412' and on the formation of the '660' and '610' intermediates. The results demonstrate that the full photocycle of bacteriorhodopsin can be observed in thin purple membrane layers even at the lowest hydration state and that the amount of absorbed water is rate limiting for the molecular process of the cycle.

Purple membranes were isolated from *H. halobium* (mutant NRL R<sub>1</sub>M<sub>1</sub>)<sup>5</sup>. Thin layers of purple membrane were prepared by drying concentrated suspension (3.5 × 10<sup>-4</sup> M) of the purple membrane in water (pH 7.2), on a glass slide. The drying was performed at atmospheric pressure, 45% relative humidity and 25 °C. The average thickness of the preparations was 1–3 µm as determined by scanning electron microscopy. Variable degrees of hydration of the purple membrane were obtained by equilibrating the samples with different relative air humidities produced by saturated salt solutions<sup>14</sup>. The glass slide with the preparations was inserted in a 1 × 1 cm cuvette and incubated in a desiccator at the required specific humidity for about 24 h. Before measurement the cuvette was immediately sealed within the desiccator with a Teflon plus Parafilm cover. The limit of dryness, obtained by drying the membrane at 10<sup>-3</sup> torr for approximately 4 h, was defined as the 0% relative humidity state.

Flash photolysis spectroscopy of thin-layer preparations of purple membrane show that bacteriorhodopsin undergoes a complete photocycle, involving the same intermediates characteristic of suspended purple membrane fragments in water. But the decay time of the '412' intermediate as well as the formation of '660' and '610' intermediates are determined by the specific hydration state of the membrane. Preparations equilibrated with 94% relative humidity show the same kinetics and

include the same transients as in purple membrane fragments suspended in water (Table 1). The relaxation times for '412' decay are slowed down by lowering the hydration state of the preparation (Fig. 1). The kinetics of '412' decay are analysed in terms of the sum of two or three exponentials (Fig. 2). The relaxation times and their relative amplitudes, at different hydration states, are given in Table 2. The initial values for the relaxation times and their corresponding amplitudes were obtained first graphically and then were optimised by a least square program (Harwell Subroutine Library, VCO5A). Essentially the same results were obtained when the initial values were calculated by a computer program (K. H. Mueller and T. Plessner, unpublished), within the frame of a non linear approximation<sup>15,16</sup>. The thermal decay kinetics of '412' could be enhanced by a factor of 15 when photobleached with 412-nm light at 0% relative humidity, in accordance with earlier experiments carried out with suspended purple membrane<sup>17</sup>.

The '660' and '610' intermediates are not formed at relative humidity lower than 90%. Moreover, the 660-nm intermediate cannot be detected at pH higher than 8 (in a solution of 4M



**Fig. 1** Oscilloscope traces obtained in flash photolytic measurements of thin purple membrane layers, kept in various hydration states. The traces represent the decay kinetics of '412' nm intermediate formed by a linearly polarised light pulse from a rhodamine 6G laser at 585 nm of 1  $\mu$ s duration. The samples were excited at right angles to the analysing light, where the plane of the thin purple membrane layers formed an angle of 45° both with the exciting and the analysis light axes. The amplified output of the photomultiplier passed through a variable RC filter to a digital scope, where the filter setting was such as to provide filter relaxation time equal to 0.05% of the oscilloscope's total time sweep width. The transient changes of '412' were recorded directly in absorbance units by the use of a lin-log converter. The flash experiments were performed with thin purple membrane equilibrated with the following relative humidities: a, 94%; b, 90%; c, 83%; d, 75%; e, 43%; f, 7%.

NaCl), or when suspending purple membrane in water-glycerol mixtures. In addition it has been shown that the formation of the '660' intermediate is temperature dependent<sup>18</sup>. Thus, the formation of this intermediate can serve as an internal probe of bacteriorhodopsin to environmental conditions, such as temperature, pH or relative humidity. The hydration effects were found to be fully reversible and each

**Table 1** Relaxation time constants ( $\tau_i$ ) of the '412', '660' and '610' intermediates

Intermediate	Purple membrane in water* ( $\tau$ , ms)	Thin purple membrane layers† ( $\tau$ , ms)
'412'	$1.7 \pm 0.6$ ( $0.21 \pm 0.13$ )	$1.8 \pm 0.2$ ( $0.3 \pm 0.07$ )
'660'	$5.0 \pm 0.8$ ( $0.79 \pm 0.13$ )	$5.4 \pm 0.9$ ( $0.69 \pm 0.07$ )
'610'	$5.4 \pm 0.4$	$5.6 \pm 0.6$
	$48 \pm 2.1$	$46.0 \pm 3.4$

The relaxation time constant is defined by  $A(t) = A_i e^{-t/\tau_i}$ , where the absorbance change is the sum of exponential terms. The relative amplitudes are given in brackets near the corresponding relaxation time constants. The experiments were done at room temperature ( $22 \pm 1^\circ\text{C}$ ).

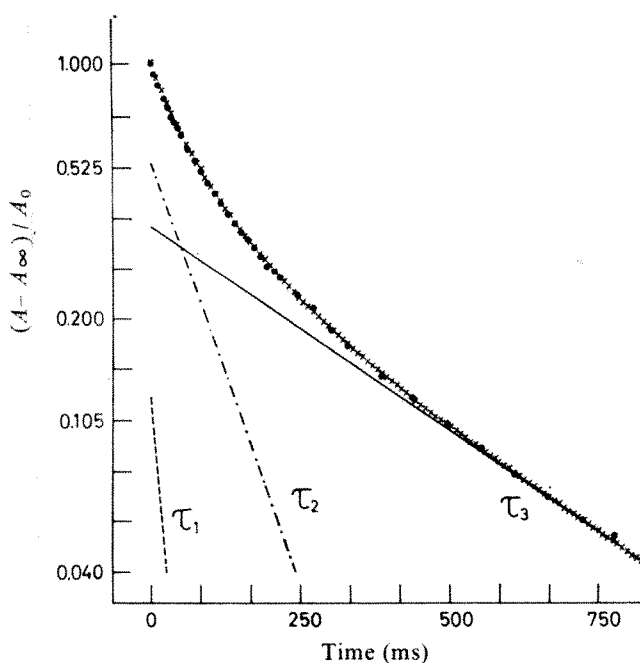
\*Suspended purple membrane in water at pH 7.2.

†Thin purple membrane preparation equilibrated with 94% relative humidity.

sample was recycled through the various hydration states several times without noticeable irreversible changes.

The results show that the amount of adsorbed water on the purple membrane determines the relaxation times of the '412' decay. The changes in hydration cause a change of the relaxation times over four orders of magnitude (Table 2). It should be added that the rate of the photoactivated formation of the intermediates in the pathway leading to '412' is only changed to a minor degree as a function of the state of hydration. The observed three relaxation times suggest that bacteriorhodopsin undergoes either a three-step conformational change along with the '412'  $\rightarrow$  BR<sup>570</sup> transition or that '412' populates three conformations which decay to that of BR<sup>570</sup>. A similar interpretation was suggested earlier for the biphasic decay of '412' (refs 17, 19).

These effects could in principle be accounted for by a rate-limited diffusion of protons due to the amount and structure of the conducting water layers on the purple membrane or by a reversible conformation change induced by the hydration



**Fig. 2** Evaluation of data from Fig. 1d. The transient absorption  $A(t)$  is expressed as a sum of exponentials by  $A(t) = A_i e^{-t/\tau_i}$ , where  $A_i$  is the amplitude of the  $\tau_i$  relaxation time constant. Closed circles represent the original data points and crosses represent the calculated data points using  $\tau_1$ ,  $\tau_2$  and  $\tau_3$  as the relaxation time constants.



**Table 2** Relaxation time constants and amplitudes of the decay kinetics of '412' intermediate in thin layer preparations of purple membrane equilibrated in various relative air humidities

Relative air humidity (%)	$\tau_1$	$\tau_2$	$\tau_3$
90	3.5 $\pm$ 0.4 ms; (0.63 $\pm$ 0.05)*	13.1 $\pm$ 1.1 ms; (0.37 $\pm$ 0.03)*	252 $\pm$ 26 ms; (0.31 $\pm$ 0.05)*
83	10.2 $\pm$ 2.4 ms; (0.18 $\pm$ 0.03)	45 $\pm$ 3.3 ms; (0.52 $\pm$ 0.02)	503 $\pm$ 111 ms; (0.33 $\pm$ 0.08)
75	30.5 $\pm$ 7.0 ms; (0.11 $\pm$ 0.04)	124 $\pm$ 33 ms; (0.56 $\pm$ 0.08)	10.1 $\pm$ 3.0 s; (0.28 $\pm$ 0.06)
43	244 $\pm$ 100 ms; (0.30 $\pm$ 0.06)	1.25 $\pm$ 0.4 s; (0.42 $\pm$ 0.04)	28 $\pm$ 8 s; (0.36 $\pm$ 0.09)
10	0.9 $\pm$ 0.2 s; (0.28 $\pm$ 0.11)	4.0 $\pm$ 1.8 s; (0.35 $\pm$ 0.06)	154 $\pm$ 21 s; (0.40 $\pm$ 0.4)
0	2.3 $\pm$ 0.7 s; (0.28 $\pm$ 0.03)	15.7 $\pm$ 4.5 s; (0.32 $\pm$ 0.03)	

\*Amplitudes are shown in parentheses.

degree of the purple membrane. The possibility of a diffusional rate-limited proton transfer could be disregarded, however, as it would have imposed a continuous spectrum of relaxation times which is inconsistent with the observed data. The possibility of extreme pH changes due to changes in the amount of adsorbed water can be ruled out, since no spectral shifts of the absorption spectrum of the thin purple membrane layers were observed at different hydrations where a blue shift of the spectrum is expected at high pH and a red shift at low pH (ref. 20 and our unpublished data). A contribution of surface electrical potential changes of the purple membrane suspended in water was analysed by using high ion concentrations (1–4M NaCl) and increasing the pH up to 9. In this extreme case the decay of the '412' was only slowed down by a factor of 50.

Although changes in hydration were shown to control the conformation of proteins<sup>21</sup>, X-ray diffraction studies<sup>4</sup> of wet and dry specimen of the purple membrane have not shown any significant differences, possibly due to the fact that local changes might escape the sensitivity of the method applied with a 7-Å resolution. Indeed, hydration-induced conformational changes might be expected to occur in much smaller dimensions. Hydration can act either on the immediate environment of the retinal or through inducing a conformational change in the protein which is then transmitted to the retinal-protein interaction region. A possible induced conformational change may also involve an increased formation of internal hydrogen bonding on dehydration, competing for proton transfer, or a conformational induced pK shift of the terminal amino acids. It should be noted that the dehydration process may bring about a large change in the dielectric constant in the vicinity of the proton acceptor. Such a change can shift the pK by two to four units<sup>22</sup> and thus change the kinetics by orders of magnitude. Variation of the hydration state of the purple membrane may change the microviscosity of the lipids or even bring to phase separation. The influence of the lipid-protein interaction on the photocycle must be of a secondary importance, however, because no significant difference was found in the kinetics and in proton uptake of proteoliposomes prepared from lipid-depleted protein and from native purple membrane<sup>23</sup>. The conformation change is further supported by the observation of a decoupling of the *cis-trans* isomerisation process as a function of hydration<sup>24</sup>, as well as by the photochemical bleaching of the '412' intermediate even at the lowest humidity state. In addition, dehydration of vertebrate rhodopsin was found to stop the meta I  $\rightarrow$  meta II transition<sup>25</sup>. Thus in both bacteriorhodopsin and in rhodopsin those conformational changes which involve both protonation-deprotonation reactions and large enthalpy change are those which are most effected by dehydration.

These results stress the importance of the state of hydration in bacteriorhodopsin, presumably as a conformation-controlling event, which might be of general significance for energy transducing membranes especially in the case where deprotonation and protonation processes, coupled to conformation changes are an inherent part of the energy transformation process. The results also indicate that the photochemical reaction is independent from the state of the bulk

solution and is only influenced by the microenvironment of the reaction system which operates in a state of quasi isolation.

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- Oesterheld, D. & Stoekenius, W. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2853–2857 (1973).
- Hildebrand, E. & Dencher, N. *Nature* **257**, 46–48 (1975).
- Oesterheld, D. & Stoekenius, W. *Nature new Biol.* **233**, 152–155 (1971).
- Henderson, R. & Unwin, P. N. T. *Nature* **257**, 28–32 (1975).
- Oesterheld, D., Muntzen, M. & Schumann, L. *Eur. J. Biochem.* **40**, 453–463 (1973).
- Kung, M. C., Devault, D., Hess, B. & Oesterheld, D. *Biophys. J.* **15**, 907–911 (1975).
- Lozier, H., Bogomolni, R. A. & Stoekenius, W. *Biophys. J.* **15**, 955–962 (1975).
- Goldschmidt, C. R., Ottolenghi, M. & Korenstein, R. *Biophys. J.* **16**, 839–843 (1976).
- Chance, B., Porte, M., Hess, B. & Oesterheld, D. *Biophys. J.* **15**, 907–911 (1975).
- Korenstein, R., Sherman, W. V. & Caplan, S. R. *Biophys. Struct. Mechanism* **2**, 267–276 (1976).
- Oesterheld, D. & Hess, B. *Eur. J. Biochem.* **37**, 316–326 (1973).
- Sperling, W., Carl, P., Rafferty, C. N. & Dencher, N. A. *Biophys. Struct. Mechanism* **3**, 79–94 (1977).
- Racker, E. & Stoekenius, E. *J. biol. Chem.* **249**, 662–663 (1974).
- Wexler, A. & Hasegawa, S. *J. Res. natn. Bur. Stand.* **53**, 19–26 (1954).
- Meinardus, G., Schwedt, D. *Archs. Rat. Mech.* **297**–326 (1964).
- Rice, J. R. *J. Soc. Industr. appl. Math.* **10**, 149–161 (1962).
- Hess, B. & Kuschmütz, D. *FEBS Lett.* **74**, 20–24 (1977).
- Sherman, W. V., Korenstein, R. & Caplan, S. R. *Biochim. biophys. Acta* **430**, 454–458 (1976).
- Eisenbach, M., Bakker, P., Korenstein, R. & Caplan, S. R. *FEBS Lett.* **71**, 228–231 (1976).
- Lozier, R. H., Niederberger, W., Bogomolni, R. A., Hwang, S. & Stoekenius, W. *Biochim. biophys. Acta* **440**, 545–556 (1976).
- Lewin, S. in *Displacement of Water and its Control of Biochemical Reactions* 99–233 (Academic, London and New York, 1974).
- Gutfreund, H. in *Enzymes: Physical Principles* (Wiley-Interscience, New York, 1972).
- Happe, M. & Overath, P. *Biochem. biophys. Res. Commun.* **72**, 1509–1511 (1976).
- Korenstein, R. & Hess, B. *FEBS Lett.* (in the press).
- Wald, G., Durell, J. & George, C. C. *S. Science* **111**, 179–181 (1950).

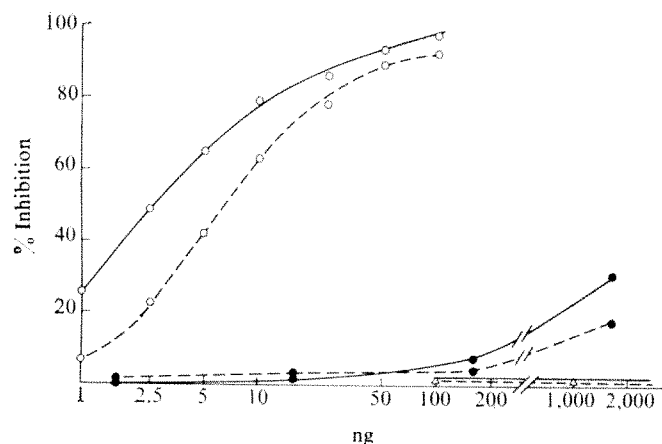
## Detection of carcinogen-DNA adducts by radioimmunoassay

COVALENT binding of carcinogen to nucleic acids is believed to be an essential component of the carcinogenic process<sup>1</sup>, so it is desirable to have highly sensitive and specific methods for detecting such adducts in cells and tissues exposed to known and suspected carcinogens. We describe here a radioimmunoassay (RIA) capable of detecting nanogram amounts of DNA adducts resulting from the covalent binding of the carcinogen *N*-2-acetylaminofluorene (AAF) and its activated derivative *N*-acetoxy-AAF (*N*-Ac-AAF) are potent carcinogens<sup>2</sup> and mutagens<sup>3,4</sup>, and transform cells in culture<sup>5,6</sup>. DNA obtained from rat liver following *in vivo* exposure to AAF, and DNA exposed *in vitro* to *N*-Ac-AAF contain as the major (80%) adduct *N*-(deoxyguanosin-8-yl)-acetylaminofluorene (dG-8-AAF)<sup>7–9</sup> and a minor (20%) adduct 3-(deoxyguanosin-5-yl)-acetylaminofluorene (dG-*N*-2-AAF)<sup>10</sup>. These two types of modification produce markedly different conformational effects on the DNA helix<sup>2,11,12</sup>. The major adduct, recognised by

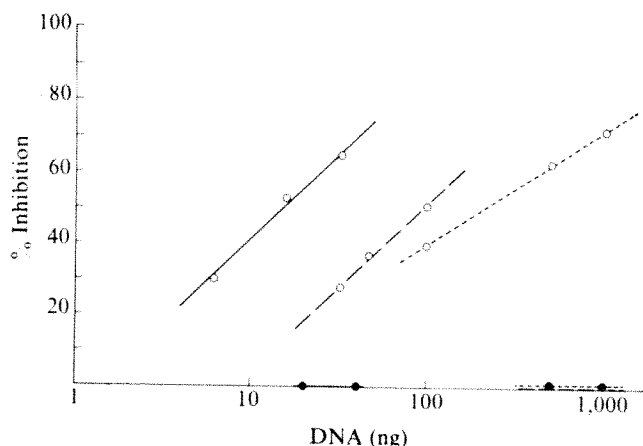
single strand-specific nucleases *in vitro*<sup>13,14</sup> and DNA repair enzymes *in vivo*<sup>9,15</sup> was used as the immunogen in this study.

Guanosine (Sigma) and *N*-Ac-AAF (Midwest Research Institute), each at 4.6 mM in 30% ethanol, were incubated for 24 h at 37 °C. The resultant *N*-(guanosin-8-yl)-acetylaminofluorene (G-8-AAF) was separated from the reaction mixture using a 20–100% methanol gradient on Sephadex LH-20 (Pharmacia) and rechromatographed until it gave a characteristic absorption spectrum<sup>8</sup>. The purified product (9 mg) was coupled to bovine serum albumin (BSA, 35 mg, Sigma) by NaIO<sub>4</sub> oxidation followed by NaBH<sub>4</sub> reduction<sup>16</sup>, yielding 3.5 mg of covalently bound G-8-AAF ( $\Sigma_{305} = 1.5 \times 10^4$ ). Three rabbits were immunised with G-8-AAF-BSA initially in complete and then in incomplete Freund's adjuvant at weeks 0, 1, 3, 12, 15, 17 and 23. Injections of 0.3 mg hapten were given intramuscularly in the hind legs. Blood was taken from the ear veins before immunisation, at 1 and 2 months, and weekly between 2 and 5 months. All three rabbits produced high levels of antibody by 4 months when assayed by RIA.

For RIA, the tracer, <sup>3</sup>H-dG-8-AAF (6 Ci mmol<sup>-1</sup>) was synthesised from *N*-Ac-AAF and deoxyguanosine (8,5'-<sup>3</sup>H, 29 Ci mmol<sup>-1</sup>, NEN), as described above for G-8-AAF. During chromatography on Sephadex LH-20, an initial small radioactive peak, (the presumed dG-N2-AAF<sup>9,10</sup>), was discarded and the major radioactive peak (dG-8-AAF) was collected and rechromatographed twice on Sephadex LH-20. A standard curve for RIA was constructed by competing the <sup>3</sup>H-dG-8-AAF with unlabelled G-8-AAF or dG-8-AAF, both of which competed equally well. High-titre serum was diluted at least 1:300 (Fig. 1). For equilibrium conditions, <sup>3</sup>H-dG-8-AAF, antiserum and inhibitor were incubated at 37 °C for 2 h, followed by goat anti-rabbit-IgG (GAR-IgG, Miles) at 22 °C or 4 °C for a minimum of 1 h. After centrifugation the supernatant was discarded and the pellets were dissolved in 0.1 M NaOH and counted. In these conditions 5–6 ng of unlabelled standard gave a 50% inhibition of antibody-bound label. The standard curves were analysed by computer program, RIAPROG<sup>17</sup>, which confirmed the linearity of the RIA by Scatchard plot. An alternate method



**Fig. 1** Radioimmunoassay standard curve at equilibrium conditions (see text) using diluted antiserum (1:1000 in 0.01 M Tris pH 7.6) (dashed line) or purified antibody diluted 1:5 in 0.01 M Tris pH 7.6 (solid line). The reaction mixture containing 0.1 ml antibody, 0.1 ml <sup>3</sup>H-dG-8-AAF (6 Ci mmol<sup>-1</sup>, 40,000 c.p.m.) and various concentrations, in 0.1 ml of G-8-AAF (○), *N*-Ac-AAF (●) or dG (△) was incubated for 2 h at 37 °C. Goat anti-rabbit-IgG (GAR-IgG, Miles) was diluted 1:2 in water and 0.1 ml added per tube. After incubation for an additional hour at 22 °C or 4 °C, the precipitate was collected by centrifugation, dissolved in 0.2 ml 0.1 M NaOH, and counted in Aquasol-2 (NEN), in a Beckman LS-250 scintillation counter. Two to four replicates were assayed per point and values agreed within  $\pm 5\%$ . A blank of about 800 c.p.m., obtained with non-immune serum, was subtracted from each assay. Concentrations of diluted antiserum or purified antibody were chosen such that about 10,000 c.p.m. were bound in the absence of an inhibitor, and this value was taken as 100%.



**Fig. 2** RIA of *N*-Ac-AAF-modified and non-modified *M. luteus* DNA in differing physical circumstances. *M. luteus* DNA (32 mg, Miles) was heated to 100 °C for 10 min, cooled rapidly in ice and incubated with 36 mg of *N*-Ac-AAF in 17% ethanol in SSC (total volume 42 ml) for 18 h at 37 °C. The reaction mixture was extracted seven times with either, reprecipitated with 70% ethanol to a stable  $A_{260}/A_{270}$ , and collected by centrifugation (150,000g, 4 °C, 48 h). Conditions for the RIA, using G-8-AAF as standard, were as in the legend to Fig. 1. Modified (○) or unmodified (●) DNA were assayed without further treatment (---), after heating to 100 °C for 10 min (—) or after enzymatic digestion (—). The enzymatic digestion was performed as follows: samples containing 500  $\mu$ g DNA were incubated per 0.5 ml of reaction mixture, with 20 units DNase I (Worthington), 5  $\mu$ mol MgSO<sub>4</sub> and 30  $\mu$ mol sodium acetate pH 5.5, at 37 °C for 4 h. The pH was then adjusted to 8.5 with (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> and 20 units venom phosphodiesterase (VP, Worthington) and 1.5 units alkaline phosphatase (AP, BAPC, Worthington) were added to 1 ml of reaction mixture followed by incubation for 18 h at 37 °C. After boiling for 10 min to denature the enzyme protein, samples were cooled on ice, centrifuged (5,000g, 4 °C) to remove the protein precipitate and the supernatant diluted in 0.01 M Tris pH 7.6 for RIA. The actual ng of dG-8-AAF per ng DNA were determined by comparing the values for % inhibition with a simultaneously-run standard curve similar to Fig. 1. The amount of DNA was calculated from  $A_{260}$ , by diphenylamine<sup>20</sup> reaction and by a fluorescence assay<sup>21</sup>, all of which agreed within 10%.

for increasing the sensitivity of the assay is the use of non-equilibrium conditions, in which the labelled hapten is added for much shorter periods of time, thus enhancing the competition of the test compound. Antibody and unlabelled standard or inhibitor were incubated for 2 h at 37 °C and cooled to 4 °C for 10 min. Tracer was then added, followed 5 min later by GAR-IgG and samples kept at 4 °C for 45 min before centrifugation. In these conditions 1 ng of either G-8-AAF or dG-8-AAF gave a 50% inhibition.

Using the RIA at equilibrium conditions the specificity of the antiserum was tested against deoxyribonucleosides, AAF and related compounds. As shown in Fig. 1, whereas 2–5 ng of G-8-AAF produced a 50% inhibition, up to 100  $\mu$ g of dG was inactive. *N*-Ac-AAF as well as AAF and *N*-OH-AAF (data not shown) produced a slight inhibition only at a very high concentration, and up to 100  $\mu$ g of dT, dA, and dC were totally ineffective (data not shown).

Antibody specific to G-8-AAF was purified from the above antiserum by affinity chromatography. Agarose- adipic hydrazide (Miles) was coupled covalently to G-8-AAF by NaIO<sub>4</sub> oxidation<sup>18</sup>, yielding 3  $\mu$ mol G-8-AAF per ml packed Agarose. Antiserum was passed through the column and 90% of the antibody activity remained bound. Elution was accomplished either by alternate washes with 0.2 M glycine pH 2.5, and 0.01 M Tris pH 7.6, or with a 5–7 M urea gradient, increased stepwise. The purified antibody showed greater sensitivity for G-8-AAF (2.5 ng gave 50% inhibition at equilibrium) than the unfractionated antiserum, and the same specificity as the diluted antiserum with respect to nucleosides and AAF derivatives (Fig. 1). The amount of purified antibody recovered was

equivalent to 20% of the binding capacity of the starting antiserum.

To define further the specificity and sensitivity of the RIA, the G-C rich DNA from *Micrococcus luteus* was extensively modified by reaction with *N*-Ac-AAF *in vitro* as described in Fig. 2. The amount of dG-8-AAF, when determined by  $A_{305}$ , indicated that 28% of the nucleotides contained covalently-bound AAF residues. A similar high degree of modification, representing 70–80% of G substitution, has been previously reported for *M. luteus* DNA reacted with *N*-Ac-AAF<sup>19</sup>. But, when assayed initially by RIA this DNA gave values indicating a 1.6% modification (Fig. 2). If the DNA was denatured by heating before RIA, values indicated a 5.5% modification. Finally, when the DNA was hydrolysed enzymatically and the digest assayed by RIA, a 27% modification, corresponding closely to that predicted by the spectral method, was observed (Fig. 2). Thus the antibody recognises only a fraction of the dG-8-AAF residues in intact DNA but quantitative detection is obtained when the DNA is hydrolysed. The 1.6% modification observed before heat-denaturation or hydrolysis might be due to partially denatured regions or AAF binding near the ends of DNA strands. These results are consistent with evidence that most of the covalently-bound AAF residues lie inside the polynucleotide structure stacked with adjacent bases<sup>11</sup> and, therefore, presumably shielded from optimum interaction with the antibody. It is of interest that antibodies to other naturally occurring modified nucleosides recognise residues in denatured, rather than native, DNA<sup>20–23</sup>.

This RIA has also been useful in detecting the extremely low levels of carcinogen modification of DNA that occur in *in vitro* conditions. As shown in Table 1, when cultured primary mouse epidermal cells were exposed to  $10^{-4}$ – $10^{-6}$  M *N*-Ac-AAF, a concentration-dependent increase in binding of AAF to cellular DNA was detected by the RIA. The results shown were reproduced in four separate experiments, although the extent of binding varied by a factor of 2 with different batches of primary cultures. Factors contributing to this type of variability with primary epidermal cell cultures have been previously discussed<sup>24</sup>. At an exposure concentration of  $10^{-6}$  M the amount of dG-8-AAF detected in three experiments (data not shown) ranged from 3.5 to 10  $\mu$ mol dG-8-AAF per mol DNA-P. These values are similar to those reported for binding measured by the specific

activity of DNA following the exposure of mouse 3T3 cell cultures to  $10^{-6}$  M  $^{14}$ C-*N*-Ac-AAF for 1 h (ref. 25).

These results indicate that the covalent binding of a chemical carcinogen, AAF, with nucleic acids can be readily detected by RIA with a specific antiserum to the carcinogen-nucleoside adduct. The complete cross-reactivity between G-8-AAF and dG-8-AAF was to be expected since the bovine serum albumin carrier was linked through the 2' and 3' OH groups of the ribose. It is remarkable that our antiserum specifically recognises the AAF-nucleoside complex, but not free AAF, or nucleoside. This specificity is of particular importance for a carcinogen like AAF where two guanosine adducts have been described. Our antigen contained only the C-8 adduct, so it is likely that our antibody detects mainly the corresponding adduct in DNA. Studies are in progress to determine whether or not there is significant cross-reactivity with dG-N<sup>2</sup>-AAF. It is possible that each type of adduct will have immunological specificity and can be independently assayed with high sensitivity during the process of AAF carcinogenesis. The usefulness of this immunological approach may be extended to other types of studies and other classes of carcinogens. Experiments to measure removal of carcinogen by DNA repair processes are in progress, and assays for the possible covalent binding of suspected environmental carcinogens to human tissues should also be feasible.

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**Table 1** Detection of dG-8-AAF by RIA in DNA from cells exposed to *N*-Ac-AAF

Concentration of <i>N</i> -Ac-AAF	Content of dG-8-AAF			
	Exp 1		Exp 2	
	ng per 100 $\mu$ g DNA	$\mu$ mol per mol DNA-P	ng per 100 $\mu$ g DNA	$\mu$ mol per mol DNA-P
$1.0 \times 10^{-6}$ M	0.57	3.5	NT	—
$1.5 \times 10^{-6}$ M	$2.56 \pm 0.6$	$15.6 \pm 3.5$	1.6	9.7
$5.0 \times 10^{-6}$ M	5.44	33.1	$2.44 \pm 0.2$	$14.8 \pm 0.8$
$5.0 \times 10^{-4}$ M	13.40	81.5	6.28	38.2

Primary BALB/c epidermal cells were prepared from newborn mice<sup>21,26</sup>, and  $2.4 \times 10^6$  cells were plated in roller bottles (Bellco, 825 cm<sup>2</sup>). On day 3, the indicated concentration of *N*-Ac-AAF dissolved in dimethylsulphoxide (DMSO) or DMSO alone, was added to the medium for 1 h at 37 °C, after which the cells were collected and DNA isolated and purified as previously described<sup>15</sup>. The DNA was then hydrolysed enzymatically as described in Fig. 2 and duplicate aliquots containing approximately 25  $\mu$ g of DNA and 50  $\mu$ g of DNA (0.025 and 0.05 ml respectively) were assayed by RIA using non-equilibrium conditions (see text). Identical results were obtained at both DNA concentrations. A standard curve was constructed from equivalent amounts of untreated and hydrolysed BALB/c epidermal DNA to which were added known nanogram quantities of dG-8-AAF. Control DNA from cultures not exposed to *N*-Ac-AAF did not inhibit the RIA. Values listed in the table refer to DNA from one bottle of treated cells except in cases (\*) where the mean  $\pm$  the range for three bottles has been given. NT, not tested.

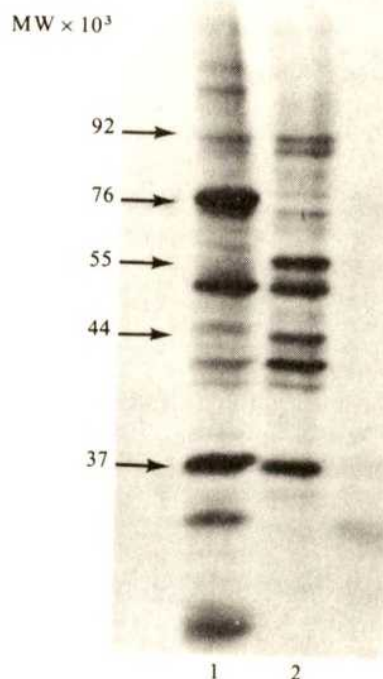
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1. Miller, J. A. *Cancer Res.* **30**, 559–576 (1970).
2. Kriek, E. *Biochim. biophys. Acta* **355**, 177–203 (1974).
3. Maher, V. M. & Wessel, J. E. *Mutat. Res.* **28**, 277–284 (1975).
4. McAnn, J., Spingarn, N. E., Kobori, J. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **72**, 979–983 (1975).
5. Yamaguchi, N. & Weinstein, I. B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 214–218 (1975).
6. Huberman, E., Donovan, P. J. & DiPaolo, J. A. *J. natn. Cancer Inst.* **48**, 837–840 (1972).
7. Miller, E. C., Juhl, U. & Miller, J. A. *Science* **153**, 1125–1127 (1966).
8. Kriek, E., Miller, J. A., Juhl, U. & Miller, E. C. *Biochemistry* **6**, 177–182 (1967).
9. Kriek, E. *Cancer Res.* **32**, 2042–2048 (1972).
10. Westra, J. G., Kriek, E. & Hittnerhausen, H. *Chem.-biol. Interact.* **15**, 149–164 (1976).
11. Weinstein, I. B. & Grunberger, D. in *Chemical Carcinogenesis*, **2** (eds Ts'0, P. O. P. & DiPaolo, J.) 217–235 (Marcel Dekker, New York, 1974).
12. Yamasaki, H., Pulkrabec, P., Grunberger, D. & Weinstein, I. B. *Cancer Res.* **37**, 3756–3760 (1977).
13. Fuchs, R. P. P. *Nature* **257**, 151–152 (1975).
14. Yamasaki, H., Leffler, S. & Weinstein, I. B. *Cancer Res.* **37**, 684–691 (1977).
15. Lieberman, M. W. & Poirier, M. C. *Cancer Res.* **33**, 2097–2103 (1973).
16. Erlanger, B. F. & Beiser, S. M. *Proc. natn. Acad. Sci. U.S.A.* **52**, 68–74 (1964).
17. Rodbard, D. *Clin. Chem.* **20**, 1255–1270 (1974).
18. Lamed, R., Levin, Y. & Wilchek, M. *Biochim. biophys. Acta* **304**, 231–235 (1973).
19. Fuchs, R. & Daune, J. *Biochemistry* **11**, 2659–2666 (1972).
20. Plescia, J. G., Braun, W. & Paleczuk, N. C. *Proc. natn. Acad. Sci. U.S.A.* **52**, 279–285 (1964).
21. Hacker, B., Van Vunakis, H. & Levine, L. *J. Immun.* **108**, 1726–1728 (1972).
22. Miller, O. J., Schmedl, W., Allen, J. & Erlanger, B. *Nature* **251**, 636–637 (1974).
23. Salomon, R., Fuchs, S., Aharonov, A., Giveon, D. & Littaur, U. Z. *Biochemistry* **14**, 4046–4050 (1975).
24. Yuspa, S. H. *et al.* *Cancer Res.* **36**, 4062–4068 (1976).
25. Amacher, D. E., Elliott, J. A. & Lieberman, M. W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1553–1557 (1977).
26. Burton, K. *Biochem. J.* **62**, 315–323 (1956).
27. Bowden, G. R., Shapas, B. G. & Boutwell, R. K. *Chem.-biol. Interact.* **8**, 379–394 (1974).
28. Yuspa, S. H. & Harris, C. C. *Expl Cell Res.* **86**, 95–105 (1974).

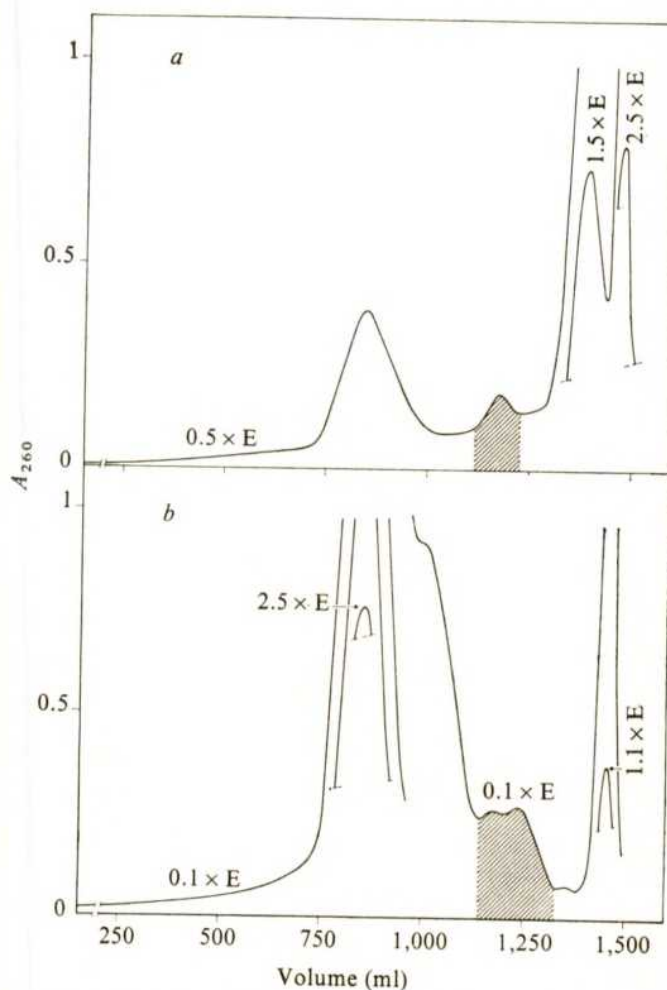


## Specific poly-A-binding protein of 76,000 molecular weight in polyribosomes is not present on poly A of free cytoplasmic mRNP

CYTOPLASM of animal cells contains mRNA associated with ribosomes (in polysomes) and mRNA free of ribosome attachment. Both classes of mRNA occur as ribonucleo-protein (mRNP) complexes<sup>1-3</sup>. The role of the untranslated pool of free mRNA (referred to as informosomes<sup>3</sup>) is uncertain. They represent the form in which mRNA exists in transit from the nucleus to the cytoplasm awaiting association with ribosomes<sup>3</sup>, and in addition the pool of free cytoplasmic mRNA contains inactive (or inactivated) and storage forms of mRNA<sup>4</sup>. It has been established that most of the polysomal mRNP complexes contain predominantly two proteins with approximate molecular weights (MW) of 49,000–52,000 and 73,000–78,000 respectively<sup>5-9</sup>. It is generally accepted that the larger of these proteins is attached to the 3' poly A tail of



**Fig. 1** Autoradiograph of a polyacrylamide gel showing patterns of proteins present in oligo dT-cellulose-purified polyribosomal and informosomal mRNP from Ehrlich ascites tumour cells. Ehrlich ascites tumour cells were labelled with <sup>35</sup>S-methionine in the presence of low concentrations of actinomycin D and ethidium bromide (0.04  $\mu\text{g ml}^{-1}$  and 1  $\mu\text{g ml}^{-1}$ , respectively) as described previously<sup>13</sup>. Polyribosomes were pelleted through a 2 M sucrose layer containing 0.5 M KCl and free cytoplasmic mRNP was pelleted from the post-ribosomal supernatant in the presence of 0.5 M KCl by a 24-h centrifugation at 200,000g. The polyribosomes and informosomal mRNP pellet were suspended in a 200 mM NaCl, 50 mM Tris-HCl, pH 7.8, 10 mM EDTA solution containing 0.2% Nonidet-P40 and applied onto an oligo dT-cellulose column. Elution of the adsorbed mRNP particles was as described by Lindberg and Sundquist<sup>7</sup>. The formamide eluted mRNP particles were analysed by electrophoresis in sodium dodecylsulphate (SDS) on 7–18% polyacrylamide gradient gels<sup>10</sup>. Channel 1, <sup>35</sup>S-labelled proteins present in polyribosomal mRNP. Channel 2, <sup>35</sup>S-labelled proteins present in informosomal mRNP. The indicated MWs were extrapolated from the positions of marker proteins (not shown): phosphorylase b (MW 92,000), bovine serum albumin (68,000), ovalbumin (45,000),  $\alpha$  A<sub>2</sub>-crystallin (20,000) and cytochrome c (12,000).



**Fig. 2** Zonal centrifugation profiles of EDTA-dissociated polyribosomes (a) and of informosomes (b) from rabbit reticulocytes. Polyribosomes were isolated from rabbit reticulocyte lysates and, after washing with 0.5 M KCl, were suspended in 10 mM Tris-HCl, pH 7.4, containing 30 mM EDTA. The post-polysomal supernatant of the lysate was centrifuged for 24 h at 78,000g. The pellet, containing the informosomal mRNP was suspended in 10 mM Tris-HCl, pH 7.4. About 2,000  $A_{260}$  units of each sample (EDTA-treated polyribosomes and informosomes) were applied onto 0–42% (w/w) zonal sucrose gradients in 10 mM Tris-HCl, pH 7.4. The mixing volume was 750 ml and overlayer and sample volumes were 550 and 50 ml, respectively. 180 ml of the gradient was used in the overlayer. Centrifugation was carried out at 35,000 r.p.m. for 21 h at 4 °C using the IEC B29 rotor. The gradient was analysed at 260 nm (2-mm flow cell) with a continuous monitoring system and the part of the gradient containing the mRNP particles (hatched area) was pooled.

mRNA<sup>10-12</sup>. We present evidence here that the poly A segments of non-polysomal mRNA of Ehrlich ascites tumour cells and of rabbit reticulocytes—in contrast to the polysomal poly A segments<sup>10-13</sup>—do not contain the 76,000 MW protein.

Ehrlich ascites tumour cells were labelled with <sup>35</sup>S-methionine in the presence of low concentrations of actinomycin D and ethidium bromide. In these conditions the proteins present in mRNP complexes were labelled whereas little radioactivity was incorporated in the proteins of high salt washed polyribosomes<sup>13,14</sup>. The oligo dT-cellulose procedure of Lindberg and Sundquist<sup>7</sup> was used to purify the poly A-containing mRNP present in polyribosomes and informosomes. The 50% formamide eluate was analysed by sodium dodecylsulphate-polyacrylamide gel electrophoresis<sup>15</sup> followed by scintillation autoradiography. The result is shown in Fig. 1. The high degree of similarity between the protein patterns of both classes of mRNP particles is





**Fig. 3** Electrophoretic analysis of the products of *in vitro* translation of purified informosomal 15S mRNP and 9S polysomal and informosomal mRNA from rabbit reticulocytes. A cell-free system from Ehrlich ascites tumour cells growing in suspension culture<sup>13</sup> was prepared as described by Hunt<sup>25</sup> and treated with micrococcal nuclease as described by Pelham and Jackson<sup>26</sup>. Purified polysomal and informosomal 9S mRNA and 15S informosomal mRNP were added to the incubation mixture (25  $\mu$ l) containing 15  $\mu$ l Ehrlich ascites extract and 2  $\mu$ l nuclease treated 0.5 M KCl wash of rabbit reticulocyte polyribosomes (initiation factors). Haemin (25  $\mu$ M) and other ingredients in the concentration described previously<sup>27</sup> were added. Incubation was for 1 h at 30 °C. Part of the incubation mixture was boiled in SDS-sample buffer and loaded onto 13% (w/v) slab gels in a buffersystem described by Weber and Osborn<sup>28</sup>. Slab gels were prepared for scintillation autoradiography as described before<sup>29</sup>. Channel 1, products synthesised by polyribosomal 9S mRNA. Channel 2, product synthesised by mRNA isolated from purified informosomal mRNP. Channel 3, product synthesised by purified informosomal mRNP. Channel 4, labelled  $\alpha$ - and  $\beta$ -globin markers.

apparent. But, the predominant 76,000 component present in polysomal mRNPs was absent in newly-synthesised informosomal mRNPs. Exhaustive degradation of informosomes from these cells with ribonucleases released poly A-protein complexes containing stretches of poly A of about 90–110 adenyl residues and a predominant 84,000 MW protein<sup>16</sup>. (In a Laemmli gel<sup>15</sup> as has been used in this work this protein was visible as a double band around 89,000 MW (Fig. 1, slot 2).) The fact that the poly A segments of these newly-synthesised mRNPs were considerably longer than the polysomal poly A segments in these cells (about 70 adenyl residues<sup>13</sup>) is an indication that we were dealing with young 'transit' mRNPs in contrast to old inactive forms of mRNPs with smaller poly A segments<sup>17–20</sup>.

To establish whether the old inactive forms of informosomal mRNPs also lack the specific poly A-binding protein of 76,000 MW, polysomal and informosomal mRNP were isolated from rabbit reticulocytes. These cells synthesise predominantly the  $\alpha$  and  $\beta$  chains of haemoglobin which are encoded by a 9S mRNA. Another characteristic of these cells is that the free cytoplasmic 9S mRNA primarily codes for the  $\alpha$  globin chain<sup>21,22</sup>. It represents mainly old mRNA characterised by a relatively small poly A segment<sup>17</sup>.

Reticulocyte polyribosomes and informosomes were isolated as described in Fig. 2 legend. The polysomal mRNP sedimented as a 15S particle (Fig. 2a), while the free cytoplasmic mRNP had S-values ranging from 15 to 20S (Fig. 2b). Both classes of particles contained 9S mRNA that

could be adsorbed on oligo dT-cellulose and eluted with a low salt buffer as described by Aviv and Leder<sup>23</sup>. The presence of a poly A tail in the informosomal mRNP was further established by hybridisation with <sup>3</sup>H-poly U (ref. 24) (data not shown).

Translation of the polysomal and informosomal mRNP and their respective mRNA was performed in a cell-free system prepared from Ehrlich ascites tumour cells<sup>25</sup> treated with micrococcal nuclease as described by Pelham *et al.*<sup>26</sup>. Figure 3 shows that polysomal 9S mRNA coded for  $\alpha$  and  $\beta$  globin, a result that is in agreement with various earlier reports. The major product synthesised by the informosomal mRNP and its 9S mRNA was  $\alpha$  globin (slot 3 and 2, respectively). The fact that free cytoplasmic mRNA (mRNP) from rabbit reticulocytes coded primarily for the  $\alpha$  globin chain has been described earlier<sup>21,22</sup> and corroborated the assumption that our 15–20S mRNP preparation was not significantly contaminated by polysome derived mRNA (or mRNP). The observation of Civelli *et al.*<sup>30</sup> that duck erythroblast informosomes are not translated (in a wheat germ cell-free system), suggests that unknown components in the cell-free system are required for translation of these particles.

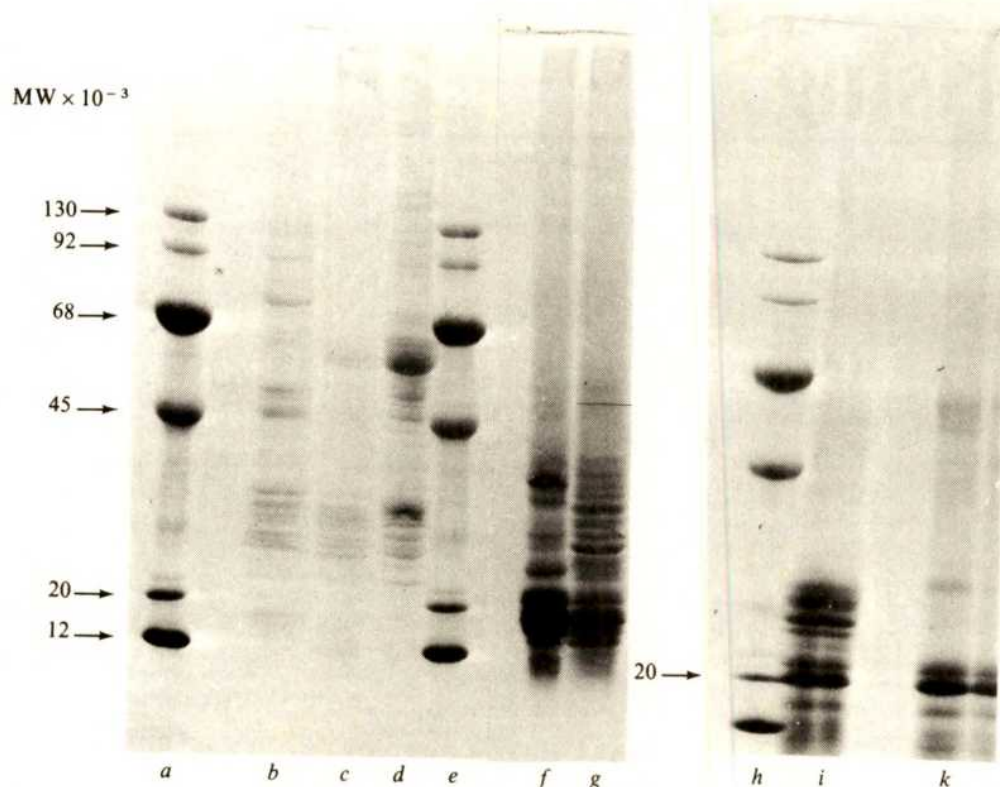
The protein composition of the 15S polysomal and 15–20S informosomal mRNP particles is shown in Fig. 4. Molecular weights were calculated using a series of marker proteins with known MW. In the polysomal mRNP the 76,000 MW protein and two proteins around 50,000 MW were prominent. A series of about six bands between 24,000 and 34,000 MW (four of them more prominent) was also a characteristic feature of the mRNP composition. The latter series of six bands was also present in informosomal mRNP. It is clear, however, that the 76,000 MW protein is not present although the informosomal mRNA contains a 3' poly A tail (see above). The protein bands around 58,000 MW (consisting of at least three proteins as could be seen in more clearly separated electrophoresis patterns) visible in slot D were removed when 15–20S informosomes were washed with 0.5 M KCl. The particles then were converted into predominantly 15S mRNP particles containing  $\alpha$  9S mRNA and the series of six bands which were probably proteins more firmly attached to the mRNA (slot i).

We conclude that poly A-containing informosomal mRNA in Ehrlich ascites tumour cells and in rabbit reticulocytes do not contain the 76,000 MW protein which in these and most other cells is associated with the poly A of polysomal bound mRNA. In view of the fact that the size of the polyadenylate segment of mRNA shortens when the message ages<sup>17–20</sup> we suggest that both poly A and the protein associated with it are degraded during ageing of mRNA. Newly-synthesised mRNA not associated with ribosomes (for example in Ehrlich ascites tumour cells) has a protein of MW 84,000–89,000 attached to its relatively long poly A segment<sup>16</sup>, whereas a 76,000 MW is attached to the poly A of mRNA in the translational phase. Later on, the mRNA (for example the informosomal  $\alpha$ -globin 9S mRNA) is released into the pool of untranslated mRNA. In this phase only low-MW proteins, if any, are attached to the shortened poly A segments. It is tempting to speculate that the loss of the 76,000 MW protein and shortening of the poly A tail are cooperative processes which are closely related to the functioning and/or stability of the mRNA.

During the preparation of this manuscript Scherrer's group<sup>31</sup> published the protein composition of informosomal mRNP from duck erythroblasts. In agreement with our observations they also found no 76,000 MW protein associated with poly A-containing informosomal mRNP.

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**Fig. 4** Analysis of proteins present in purified polysomal and informosomal mRNP particles from rabbit reticulocytes. mRNP particles were dissolved in SDS-sample buffer, boiled and loaded on to a 7–18% polyacrylamide gradient gel containing SDS<sup>15</sup>. Channels *a*, *e*, *h*, marker proteins:  $\beta$ -galactosidase (MW 130,000), phosphorylase b (92,000), bovine serum albumin (68,000), ovalbumin (45,000),  $\alpha$ A<sub>2</sub>-crystallin (20,000), cytochrome *c* (12,000). *b*, Polysomal 15S mRNP. *c*, Informosomal mRNP after a 0.5 M KCl wash (see also *i*). *d*, Informosomal mRNP before the 0.5 M KCl wash. *f*, EDTA-treated 40S ribosomal subunits. *g*, EDTA-treated 60S ribosomal subunits. *i*, Informosomal mRNP after a 0.5 M KCl wash (double band at 20,000 is carrier  $\alpha$ -crystallin). *k*, 0.5 M KCl wash of informosomal mRNP (double band at 20,000 carrier is  $\alpha$ -crystallin).

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- Williamson, R. *FEBS Lett.* **37**, 1–6 (1973).
- Greenberg, J. R. *J. Cell Biol.* **64**, 269–288 (1975).
- Spirin, A. S. *Eur. J. Biochem.* **10**, 20–35 (1969).
- Scherrer, K. *Cold Spring Harb. Symp., Symp. quant. Biol.* **35**, 539–554 (1970).
- Bryan, R. N. & Hayashi, M. *Nature new Biol.* **244**, 271–274 (1973).
- Greenberg, J. R. *J. molec. Biol.* **108**, 403–416 (1977).
- Lindberg, U. & Sundquist, B. *J. molec. Biol.* **86**, 451–468 (1974).
- Barrieux, A., Ingraham, H. A., Nystul, S. & Rosenfeld, M. G. *Biochemistry* **15**, 3523–3528 (1976).
- Liautaud, J. P., Setyono, B., Spindler, E. & Köhler, K. *Biochim. biophys. Acta* **425**, 373–383 (1976).
- Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **70**, 924–928 (1973).
- Schwartz, H. & Darnell, J. E. *J. molec. Biol.* **104**, 833–857 (1976).
- Kish, V. M. & Pederson, T. *J. biol. Chem.* **251**, 5888–5894 (1976).
- Janssen, D. B., Counotte-Potman, A. D. & Van Venrooij, W. J. *Molec. Biol. Rep.* **3**, 87–95 (1976).
- Van Venrooij, W. J. & Janssen, A. P. M. *Eur. J. Biochem.* **69**, 55–60 (1976).
- Laemmli, U. K. *Nature new Biol.* **227**, 680–686 (1970).
- Van Venrooij, W. J., Jansen, R. T. P. & Janssen, D. B. *Biochem. Soc. Trans.* **5**, 662–663 (1977).
- Marbaix, G., Huez, G., Nokin, P. & Cleuter, Y. *FEBS Lett.* **66**, 269–273 (1976).
- Sheiness, D. & Darnell, J. E. *Nature new Biol.* **241**, 265–268 (1973).
- Nokin, P., Huez, G., Marbaix, G., Burny, A. & Chantrenne, H. *Eur. J. Biochem.* **62**, 509–517 (1976).
- Merkel, C. G., Gordon Wood, T. & Lingrel, J. B. *J. Biol. Chem.* **251**, 5512–5515 (1976).
- Bonanou-Tzedaki, S. A., Pragnell, I. B. & Arnstein, H. R. V. *FEBS Lett.* **26**, 77–82 (1972).
- Jacobs-Lorena, M. & Baglioni, C. *Eur. J. Biochem.* **35**, 559–565 (1973).
- Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
- Slegers, H. & Kondo, M. *Nucleic Acids Res.* **4**, 625–639 (1977).
- Hunt, L. *Virology* **70**, 484–492 (1976).
- Pelham, H. R. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247–256 (1976).
- Saldeen, M. H. L., Gielkens, A. L. J. & Bloemendal, H. *Biochim. biophys. Acta* **425**, 208–219 (1976).
- Weber, K. & Osborn, M. *J. biol. Chem.* **244**, 4406–4412 (1969).
- Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83–88 (1974).
- Civelli, O., Vincent, A., Buri, J. F. & Scherrer, K. *FEBS Lett.* **72**, 71–76 (1976).
- Vincent, A., Civelli, O., Buri, J. F. & Scherrer, K. *FEBS Lett.* **77**, 281–286 (1977).

## Corrigendum

In 'Voltage signal of photoreceptors at visual threshold' by G. L. Fain, A. M. Granda and J. H. Maxwell (*Nature* **265**, 181; 1977), the ordinate label of Fig. 1 should read:  $\log \Delta I_T$  ( $\mu\text{J cm}^{-2} \text{ flash}^{-1}$ ). Likewise, the abscissa label of Fig. 1 should read:  $\log \Delta I_T$  ( $\mu\text{J cm}^{-2} \text{ s}^{-1}$ ). This is simply a labelling error and has no effect on any numbers in the text. The authors are grateful to Dr G. S. Wasserman of Purdue University for pointing out this error.

## Errata

In the article 'Corrected age of the Pliocene/Pleistocene boundary' by B. U. Haq, W. A. Berggren & J. A. Van Couvering, *Nature* **269**, p. 483, the legend to Fig. 5 should read . . . Pliocene/Pleistocene calcareous plankton biochronology in deep-sea cores and estimated chronostratigraphic position of Calabrian sequences. The extinction of *Discoaster brouweri* occurs at about 1.8 Myr (\*) in one of the cores studied (V12–18). The upper limit of this species, as shown here, may thus be somewhat younger than the actual extinction datum, due to reworking at the depositional interface. ○, Atlantic only.

In the letter 'Corollary discharge to cockroach giant interneurons' by F. Delcomyn, *Nature* **269**, p. 160, line 17 in paragraph 4 should read . . . When a strong stream of air is suddenly turned on a quiet . . .

In the letter 'Direct measurements of secondary currents in river bends' by J. C. Bathurst, C. R. Thorne and R. D. Hey, *Nature* **269**, p. 504, line 2 in paragraph 6 should read . . . angle  $\phi$  to the longstream axis.  $\phi$  defines the vector of the . . .

# matters arising

## How did barium titanate particulates stick together in the Nebula?

MASUDA and Tanaka<sup>1</sup> in their recent letter have raised this question. Seeking explanation in the ferroelectric (FE) properties of BaTiO<sub>3</sub> we should ask now more specifically whether FE particles, similar to magnetic particles<sup>2</sup>, may provide preferred nuclei for accretionary processes.

A FE particle acquires charge densities ( $\sim 10^{-4}$  C cm<sup>-2</sup>) much greater than is found with ordinary electrostatic charging, for example, that arising from the termination of crystal structure in unsaturated and dangling bonds which become rapidly screened by adsorption. To produce the kind of homogeneous polarisation which FE particles develop at their Curie temperature  $T_c$  (393 K for BaTiO<sub>3</sub>) in an ordinary dielectric would require fields of  $10^6$ – $10^8$  V cm<sup>-1</sup>. The distorted surface layers observed on sub- $\mu$ m FE particles<sup>3</sup> indicate that the particles do not find it easy to screen their surface charges by adsorption or internal electrical conduction. Moreover,  $T_c$  of particles  $< 1 \mu$ m may exceed 700 K. We may thus expect the potential corresponding to a FE particle to be much greater than the 1–30 V considered feasible for normal electrostatically charged interplanetary grains<sup>4,5</sup>.

The potential energy of a small uniformly polarised BaTiO<sub>3</sub> crystal, represented by a doublet  $p_i$ , outside a complex formed by a collection of many uniformly polarised crystals and additional charge distributions corresponding to non-FE constituents, is determined by the normal component  $p_n$  at the surface of the complex. To determine the capture cross section<sup>6</sup> we consider a complex of radius  $r$  and surface polarisation  $p_n$  moving with velocity  $u$ . A doublet  $p_i$  with mass  $m_i$  and velocity  $v$  makes its closest approach at distance  $R$  from the centre of the complex and is captured in a grazing orbit. On the supposition of conservation of energy and momentum we obtain for the ratio  $K = (\pi R^2)/(\pi r^2)$

$$K \approx 1 + \frac{p_i p_n (1 + 3 \cos^2 \theta)^{1/2}}{4 \pi r^3 m_i v^2}$$

Taking  $p_i$  and  $p_n$  to be of the order of  $\approx 5 \times 10^{-5}$  C and  $v \approx 1.5$  cm s<sup>-1</sup> gives  $K \approx 5 \times 10^7$  for a 0.15  $\mu$ m particle.

The micrographs, presented by Masuda and Tanaka<sup>1</sup>, show a large number of small composite particulates with a narrow size distribution around 0.15  $\mu$ m and two conspicuous clusters of  $\sim 5 \mu$ m. If we were to interpret the latter as records of enhanced

capture cross sections of BaTiO<sub>3</sub> in the early environment within the nebula, then these would suggest enhancement factors between  $1.7$  and  $10^3$  and  $\sim 10^7$  depending on the size of the initial condensates.

While an enhanced capture cross section may explain why FE particles will stick together when available, it does not explain the high Ba abundances found in eucrites and in the Allende meteorite<sup>7,8</sup> as such. In view of the wide gap between the condensation temperature of  $\sim 1,680$  K and  $T_c$  those high BaTiO<sub>3</sub> concentrations which lie well above statistical fluctuations suggest selective spatial separation and enrichment. On account of the very high dielectric constant, even above  $T_c$ , the radiation pressure on BaTiO<sub>3</sub> condensates must have been greater than for most other particles of similar density and cross section. (Reflectivity coefficient for carbonaceous chondrites is  $\sim 0.04$ – $0.07$ , (ref. 8) for BaTiO<sub>3</sub>  $\sim 1$ .) For particles of  $\sim 0.1 \mu$ m the force due to radiation pressure outweighs by far the gravitational attraction. Assuming the latter to be balanced by the centrifugal force in the orbital motion, the spatial distribution of condensates will be determined primarily by the radiation pressure and the Lorentz forces due to a solar or interplanetary magnetic field<sup>10,11</sup>.

Considering conditions at 4 a.u. and assuming a moderate particle potential of 100 V in a uniform magnetic field of  $5 \times 10^{-5}$  gauss we obtain, in Table 1, for the forces due to radiation ( $f_R$ ), gravitation ( $f_G$ ) and Lorentz forces ( $f_L$ ) the following values for particles of 0.125, 1, and 5  $\mu$ m.

Table 1 Forces acting on FE particles

	0.125 $\mu$ m ( $\mu$ N)	1 $\mu$ m ( $\mu$ N)	5 $\mu$ m ( $\mu$ N)
$f_R$	$2.6 \times 10^{-14}$	$1.8 \times 10^{-12}$	$4.5 \times 10^{-11}$
$f_G$	$1.7 \times 10^{-14}$	$1.1 \times 10^{-11}$	$1.4 \times 10^{-9}$
$f_L$	$8.5 \times 10^{-13}$	$5.4 \times 10^{-12}$	$1.3 \times 10^{-11}$

We may thus infer that FE particles up to 1  $\mu$ m may settle out preferentially towards the inner part of the solar nebula. As particles aggregate into clusters of 5  $\mu$ m, however, the radiation pressure wins out and depending on growth and ablation, larger particles are thus likely to equilibrate at 4 a.u. or to be swept out to greater distances.

At distances  $\geq 1.75$  a.u. with grain temperatures  $< 300$  K a BaTiO<sub>3</sub> grain should be in its orthorhombic or rhombohedral modification. It would, therefore, be interesting to determine the crystal structure

of BaTiO<sub>3</sub> grains in the Allende meteorite. Moreover, the small dispersed particles as well as the larger clusters should exhibit FE polarisation and hysteresis<sup>11–13</sup> and it would thus be instructive to determine their *in-situ* Curie temperature. Structure and transition temperatures may give away further clues as to how BaTiO<sub>3</sub> grains came to be a vastly enriched constituent in the composite matrix forming the Allende meteorite.

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- Masuda, A. & Tanaka, T. *Nature* **267**, 23 (1977).
- Harris, P. G. & Tozer, D. C. *Nature* **215**, 1449 (1967).
- Jona, F. & Shirane, G. *Ferroelectric Crystals* 181 (Pergamon, London, 1962).
- Belton, M. J. S. *Science* **151**, 35 (1966).
- Burke, J. R. & Silk, J. *Astrophys. J.* **190**, 1 (1974).
- Spitzer, L. *Diffuse Matter in Space*, Interscience Texts on Physics and Astronomy no. 28 (Wiley, New York, 1928).
- Schneitzler, C. C. & Philpotts, J. O. in *Meteorite Research* (ed. Millman, P. M.) 206 (Reidel, Dordrecht, 1961).
- Krino, E. L. *Principles of Meteoritics* 419, 430 (Pergamon, Oxford, 1960).
- Allven, H. *On the Origin of the Solar System* (Clarendon, Oxford, 1954).
- Allven, H. *Cosmical Electrodynamics* 18 (Clarendon, Oxford, 1963).
- Timco, G. W. & Schloessin, H. H. *High Temperatures-High Pressures* **8**, 73 (1976).
- Timco, G. W. thesis, Univ. Western Ontario, (1977).
- Schloessin, H. H. & Timco, G. W. *Phys. Earth Planet. Inter.* **14**, P6 (1977).

## Diversity of deep-sea benthos

THE remarkable diversity of the deep-sea benthos is now well known, and Wolff<sup>1</sup> has contrasted two theories to explain this. One is Sanders<sup>2</sup> theory of niche specialisation, the other is Dayton and Hessler's<sup>3</sup> theory of biological disturbance, including disturbance by predators. One point that may discriminate between theories explaining diversity is that it seems that many deep-sea benthic forms have long life expectations and low reproductive rates<sup>1</sup>. I point out here that these life history characteristics are found in another quite distinct group of high diversity, which also lives in a habitat with very little seasonal change—tropical forest birds. Snow<sup>4</sup> reviews much of what is known of the population dynamics of such birds, particularly the fruit-eating ones. The life history seems to be characterised by small clutch size and yet a very high early mortality, particularly of eggs and nestlings, and a low mortality thereafter. This early mortality is caused by predators. Snow says "Once a bird has survived the early weeks of life, the tropical forest is a

much safer place to live in than the woods of temperate regions. Crises are rare: day after day passes in a peaceful, almost monotonous routine". Is it not possible that a similar pattern of life history mortality, high when very young, low thereafter, and a lack of seasonal changes, have enabled the evolution of high diversity both in the tropical forest and in the deep sea? If this is so, the critical specialisations would presumably be in minimising the early mortality in various ways, and small brood sizes may be a necessary part of these specialisations, by making detection by predators more difficult<sup>4</sup>. There would then be much less pressure for niche specialisation among the adults.

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1. Wolff, T. *Nature* **267**, 780–785 (1977).
2. Sanders, H. L. *Brookhaven Symp. Biol.* **22**, 71–81 (1969).
3. Dayton, P. K. & Hessler, R. R. *Deep-Sea Res.* **19**, 199–208 (1972).
4. Snow, D. W. *The Web of Adaptation, Bird Studies in the American Tropics* (Collins, London, 1976).

## Contemporaneity of late Cretaceous extinctions

THE dramatic extinctions at the end of the Cretaceous, both on land and in the sea, have long provoked speculation, but there is little direct evidence. Their existence is why the Cainozoic Era is distinguished from the Mesozoic, and in practice they are used in stratigraphy to make the Cretaceous–Palaeocene boundary. If the extinctions occurred at different times in different places, this practice would have to be revised.

Butler *et al.*<sup>1</sup> presented magnetostratigraphic evidence that dinosaur extinction in northern New Mexico occurred slightly later than the marine extinctions in central Italy. They seem to generalise this difference to all marine and terrestrial rock sequences, but this may be incorrect.

On the basis of extensive evidence of diverse kinds from Montana, we have been able to reconstruct ecologically the process of terrestrial extinctions there (refs 2–4 and unpublished). The time scale was ecologically very long but geologically very short ( $10^4$ – $10^5$  yr), just as in marine sequences<sup>5,6</sup>. The process, which affected marsupials almost as severely as dinosaurs but left the freshwater community entirely unaffected, was a protracted ecological succession which seems to have operated mainly by diffuse competition with placental and multituberculate mammals. This invading mammalian community<sup>7,8</sup> soon expanded worldwide to give rise to most of our familiar Cainozoic mammals.

We have predicted<sup>2,3</sup> that terrestrial extinctions farther south occurred later than those in Montana, but we were able to find<sup>2</sup> only two flimsy data relevant to

this prediction. Both were from South America and both supported the prediction. The extinctions in Montana were probably not the earliest, as dinosaurs occurred at least to about 75°N palaeolatitude<sup>2,9</sup> and we expect that dinosaurs disappeared here a little before they did near 50°N in Montana.

It would be a remarkable coincidence if the great marine and terrestrial extinctions did not have causal elements in common. We believe that these extinctions were in part synchronous but that the terrestrial extinctions moved south as the rapidly evolving placental mammals expanded out from their ancestral temperate community. Dinosaurs may well have survived into the Eocene in India<sup>2</sup>, which was an island during the critical interval.

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2. Van Valen, L. & Sloan, R. E. *Evol. Theory* **2**, 37–64 (1977).
3. Van Valen, L. & Sloan, R. E. *Proc. 24th Int. Geol. Cong.* **7**, 214 (1972).
4. Sloan, R. E. *Science* **146**, 430 (1964); in *Athlon* (ed. Churcher, C. S.) 134–154 (Royal Ontario Museum, Toronto, 1976).
5. Percival, S. F., Jr & Fischer, A. G. *Evol. Theory* **2**, 1–305 (1977).
6. Worsley, T. R. thesis Univ. Illinois.
7. Sloan, R. E. & Van Valen, L. *Science* **148**, 220–227 (1965).
8. Van Valen, L. & Sloan, R. E. *Science* **150**, 743–745; 1796 (1965).
9. Rouse, G. E. & Srivastava, S. K. *Canadian J. Earth Sci.* **9**, 1163–1179 (1972).

## Do fatty acids inhibit gibberellin-induced amylolysis?

BULLER *et al.*<sup>1</sup> reported that fatty acids inhibit gibberellin-induced amylolysis in barley endosperm. Their experiments as described do not show if the effect is on induction of amylase by gibberellin, on amylase activity, or on resistance of starch to amylase attack, although they discuss results as "the exceptional efficacy of pentanoic ( $C_5$ ) and nonanoic ( $C_9$ ) acids in overcoming the effect of GA...".

Mitchell and Zillmann<sup>2</sup> showed that fatty acids increased flour and starch pasting ability, and some years ago I measured similar effects. In the Amylograph<sup>3</sup>, with the naturally occurring enzymes inactivated<sup>4</sup>, a wheat flour had maximum paste viscosity 1,205 Brabender Units (BU). Fatty acid additions (500 mg each to the mixture of 60 g flour and 440 ml water) gave the follow-

ing increases of maximum viscosity, confirming Mitchell and Zillmann's observations:  $C_6$ , 45 BU;  $C_7$ , 85 BU;  $C_8$ , 160 BU;  $C_9$ , 125 BU;  $C_{10}$ , 10 BU and  $C_{11}$ , 5 BU.

If the flour enzymes (including amylases) were not inactivated, then  $C_8$  fatty acid had remarkably greater effect. Adding 500 mg (58  $\mu$ mol per g flour, or 7.9 mM) increased viscosity from 495 BU to 975 BU. This effect for very low amylase activity suggests that fatty acid altered starch properties and made it more resistant to enzymatic breakdown. Commercial glyceryl monostearate (GMS) has a similar effect but GMS plus  $C_8$  acid was no more effective than one component, nor did glyceryl monocaprylate strengthen starch more markedly than equimolar amounts of  $C_8$  acid or GMS.

I suggest that fatty acids about  $C_8$  can make starch more resistant to amylase degradation. Thus the effect demonstrated by Buller *et al.* could be of fatty acid making starch more resistant to amylolysis rather than an effect associated with gibberellin.

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2. Mitchell, W. A. & Zillmann, E. *Trans. Am. Ass. Cereal Chemists* **9**, 64–79 (1951).
3. Meredith, P. *Cereal Chem.* **47**, 492–500 (1960).

BULLER AND REID REPLY—We have reported that saturated fatty acids of chain length around  $C_9$  are potent inhibitors of gibberellin-triggered amylolysis in embryo-free barley half-grains<sup>1</sup>. We are aware that the efficacy of these acids "in overcoming the effect of GA" might be due either to their interfering with some aspect of the production, activation or secretion of amylolytic enzymes or to their inhibition of the amylolytic enzymes themselves. We are now investigating the biochemical mechanism(s) of fatty acid action.

In experiments carefully controlled to eliminate fatty acid effects on amylase activity in our assay systems we have observed that the fatty acids of chain lengths  $C_7$ ,  $C_8$  and  $C_9$  at  $5 \times 10^{-4}$  M completely inhibit the release of amylase activity from gibberellin-treated barley aleurone layers. This suggests that the fatty acids can interfere, directly or indirectly, with amylase 'induction' or release.

Yet fatty acids can also inhibit the activity of amylolytic enzymes. Nonanoate ( $C_9$ ) at  $10^{-3}$  M reduces by about 60% (relative to a citrate control) the



rate of production of reducing equivalents from a solution of potato starch by a crude soluble 'amylase' prepared from germinated barley (N. Harris and J. S. G. R., unpublished).

Our results to date, therefore, suggest that fatty acids of chain length around C<sub>9</sub> inhibit both the production and the activity of amylolytic enzymes. Both effects undoubtedly contributed to our earlier observations<sup>1</sup>.

Meredith's suggestion that fatty acids might interact with starch, making it less susceptible to amylolysis, adds a further variable to an already complex problem. Any observed inhibition by fatty acids of amylolytic activity could be attributed either to a fatty acid-enzyme interaction or to a fatty acid-starch interaction as proposed by Meredith. We cannot yet assess the relative importance of the two effects in our assays, and we point out that even the experimental data presented by Meredith can be reinterpreted in terms of a fatty acid-enzyme interaction.

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## An imbricate thrust model for Southern Uplands of Scotland

THE timing of the letter by McKerrrow, Leggett and Eales<sup>1</sup> is curious in that the model is not new. It was anticipated in concept by Mitchell<sup>2</sup> and in detailed demonstration by Fyfe and Weir<sup>3</sup>. In addition information given within the article includes details of the sequences seen along the British Gas pipeline, and a structural analysis from the Moffat area by Eales. The former is interesting although unexceptionable, apart from the fact that it sets aside without justification previous evidence that not all the Ordovician volcanics are of Arenig age<sup>4-6</sup>. The structural analysis is unsubstantiated but, more importantly, it ignores structural sequences already established from many other areas in the Southern Uplands and Ireland, which are supported by recent studies<sup>7,8</sup>.

The history of Cockburnland also requires clarification. Originally proposed<sup>9</sup> as the largely ophiolitic source for the Ordovician flysch of the Southern Uplands, it has been criticised as inadequate to provide the accumulated volume of sediment<sup>9</sup>. The

name was retained, but was used instead to designate the landmass uplifted in Llandovery times<sup>10</sup> south of the nascent Southern Uplands Fault, and now considered to represent the emergence of an evolving accretionary prism. Obduction of oceanic crust towards or over the Laurentian front<sup>11</sup> would create a sedimentary source adequate to provide the known volume of Ordovician flysch, thus dispelling the objection to an Ordovician Cockburnland. We consider that application of the name to the developing accretionary prism can be justified only by assuming geographical continuity with the remnants of the original structure.

Furthermore, the model prescribes progressive sedimentation of coarse clastics from north west to the south east. This must be an over-simplification, in that for at least part of the Llandovery the Moffat shale belt was bounded to north west and south east by thick sandy sequences<sup>12</sup>.

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1. McKerrrow, W. S., Leggett, J. K. & Eales, M. H. *Nature* **267**, 237-239 (1977).
2. Mitchell, A. H. G. *Nature* **248**, 747-749 (1974).
3. Fyfe, T. B. & Weir, J. A. *Scott. J. Geol.* **12**, 93-102 (1976).
4. Lamont, A. & Lindström, M. *Trans. Edinb. geol. Soc.* **17**, 60-70 (1957).
5. Walton, E. K. in *The British Caledonides* (ed Johnson, M. R. W. & Stewart, F. H.) 71-97 (Oliver & Boyd, Edinburgh, 1963).
6. Mendum, J. C. *Trans. Leeds geol. Assoc.* **7**, 261-264 (1968).
7. Floyd, J. D. thesis, Univ. St Andrews (1975).
8. Cook, D. R. thesis, Univ. St Andrews (1976).
9. Ziegler, A. J. *Geol.* **78**, 445-479 (1970).
10. McGivern, A. thesis, Univ. Glasgow (1967).
11. Church, W. R. & Gayer, R. A. *Geol. Mag.* **110**, 497-510 (1974).
12. Clarkson, C. M., Craig, G. Y. & Walton, E. K. *Trans. R. Soc. Edinb.* **69**, 313-325 (1975).

MCKERRROW, LEGGETT AND EALES  
REPLY.—The timing of our contribution was the result of work carried out in 1976 on new exposures in the British Gas pipeline. The model relating the structure of the Southern Uplands to contemporary subduction was first illustrated by Mitchell and McKerrrow<sup>1</sup>; this model was based, in part, on earlier work by Craig and Walton<sup>2</sup>, Toghill<sup>3</sup>, Kelling<sup>4</sup> and Fyfe and Weir<sup>5</sup>.

Our contribution did not imply that all the Ordovician volcanics in the Southern Uplands are of Arenig age. Those near Abington lie below Arenig cherts, while those at Coulter underlie Llandeilo cherts; the cherts in both areas may well be much younger than the basalts below them. In addition, there are other volcanics interbedded with greywackes (like those at Wrae

and Bail Hill), but these are not oceanic basalts.

The structural analysis (Eales, unpublished) was applied specifically to the incompetent Moffat Shale facies where it occurs at the base of major thrust sheets. It is only in this facies, with its classical graptolite faunal successions, that refined stratigraphy can be used as a basis for structural synthesis. Other local structural sequences (for example, Rust<sup>6</sup>; Weir<sup>7</sup>) are generally consistent with this analysis, but they lack detailed stratigraphic control.

Walton<sup>8</sup> gave the name Cockburnland to the emergent "chain stretching north-eastwards along the northern margin of the present Southern Uplands" which provide a source for the Llandeilo and Caradoc rocks of the area around Glen App. He also used the same name for the Llandovery landmass which provided sediment both northwards (to the Silurian of the Midland Valley inliers) and southwards (to the Central and Southern Belts of the Southern Uplands). The 'Cockburnland' shown in our contribution (Fig. 3) is the second of these two land areas. It is not clear whether the earliest Ordovician greywackes were derived directly from previously obducted ophiolites lying to the north of the Southern Upland Fault (as Walton and Weir suggest), or whether they were derived from previously accreted ocean floor volcanics and cherts on the inner trench wall.

Our model implies that most of the sediments in the Southern Uplands were deposited on the floor of (or on the margin of) the Iapetus Ocean. Even though coarser sediments occur in the Llandovery and Wenlock beds exposed in the Southern Belt of the Southern Uplands, this does not imply any south-east boundary to the 'Moffat Shale belt', it merely shows that coarser sediments were being deposited on the Iapetus Ocean at this time.

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1. Mitchell, A. H. G. & McKerrrow, W. S. *Bull. geol. Soc. Am.* **86**, 305-315 (1975).
2. Craig, G. Y. & Walton, E. K. *Geol. Mag.* **96**, 209-220 (1959).
3. Toghill, P. *Scott. J. Geol.* **6**, 233-242 (1970).
4. Kelling, G. Q. *J. geol. Soc. Lond.* **117**, 37-75 (1961).
5. Fyfe, T. B. & Weir, J. A. *Scott. J. Geol.* **12**, 93-102 (1976).
6. Rust, B. R. *Scott. J. Geol.* **1**, 101-133 (1965).
7. Weir, J. A. *Scott. J. Geol.* **4**, 31-52 (1968).
8. Walton, E. K. in *The British Caledonides* (eds Johnson, M. R. W. & Stewart, F. H.) 71-97 (Oliver & Boyd, Edinburgh, 1963).

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## Filling Bondi's place

PROFESSOR Sir Hermann Bondi, profiled this week (page 201), took up his post as chief scientist at the UK Department of Energy on 1 October. That the post he left at the Ministry of Defence has remained unfilled for a comparatively short while should not diminish concern over the way this and similar appointments are made.

Bondi's former post is the highest in government which any scientist can hope to achieve. Its holder has the stature to receive the most privileged information, and the access to offer independent advice, across the broadest range of issues, at the highest levels of government. To have it vacant longer than necessary is thus undesirable. Yet the Ministry of Defence has known of Bondi's departure for nearly five months—possibly longer.

The delay has a simple and not unreasonable explanation. Because the post is also the highest which can be occupied by someone from outside both political and civil service ranks, the appointee must be acceptable to those he will work with—service chiefs as well as ministers and civil servants. The choice will be further limited by the need for

a 'link man' with the scientific community who has standing and prestige and who is also willing to give up his job. And even when the right man is found, there remains the crucial matter of security clearance.

The question is whether such an important appointment, of such consequence for the public interest, should be made more openly even though—perhaps because—the number of candidates is so restricted. The argument against this is that to name names would subject potential candidates to considerable pressures in their existing jobs, and that the consequences of a failed security clearance would be fearfully embarrassing—all without much good being done. But this does not mean that public canvassing of names cannot work. Indeed, it works elsewhere, notably in the United States, where experience with it is admittedly far greater. The public interest aspect aside, the US system may even work better. If so, the question now is whether a change could be implemented smoothly in Britain without ending up with the worst of both systems. The trouble is, no one is involved in or even encouraging discussion of it. □

## New frontiers at Trieste

THE International Centre for Theoretical Physics (ICTP) at Trieste, dedicated to the support of physicists in the developing world, lives on several knife-edges. For one thing it relies on comparable contributions for its support from the international agencies UNESCO and IAEA and also on the continued financial goodwill of the Italian government and the City of Trieste. For another there are many, scientists, included, who wonder whether theoretical physics is a luxury that it is no business of the developed world to stimulate by means of courses at a very high level. Yet again, inevitably the quality of those who attend is much more variable than, say, amongst a group of students with a common background; thus the Centre will have more than its fair share of visitors who feel lost in its environment. And finally, there are certainly those who say 'if theoretical physics, why not experimental biology', and so on.

The Centre, under the guidance of Abdus Salam, has not, however, put up the shutters against criticism. Rather, it has been making moves to add new disciplines to its range of interests. Thus in the past couple of years it has held substantial courses on the teaching of physics at French-speaking universities in the developing world, on solar-energy conversion, on the physics of oceans and the atmosphere and, most recently, on the physics of the Earth. There is talk, also, of the possibility of a new experimental centre to be built on the Yugoslav/Italian border.

As the centre adds new peripheral interests to its core subjects of high-energy and nuclear physics, solid state physics and applicable mathematics, it finds itself closer to the tricky problems of science and society. Indeed the oceans and atmosphere course was stimulated by the Bangladesh floods, and the physics of the Earth course by the Friuli earthquake of last year which shook Trieste. Out of such courses can come politically relevant action, as happened last week. Students on the course were presented with lectures on earthquake-prediction research as practised in the United States, Soviet Union, Italy, China and

Japan. But the question arose, what should developing countries be doing about this? Many of the worst losses of life and property occur in the developing world; are such countries to wait supinely until systems are perfected elsewhere, and then be confronted with a very expensive package? Yet if not, most developing countries could not mount a major research initiative.

The need, then, is for a middle way which encourages scientists to keep in touch with developments and accumulate the necessary background information and which convinces politicians that there are human and economic benefits to treating the matter seriously. The centre, it emerged during discussions, could play a significant role in assisting technology transfer. The proposal was that ICTP, with its devotion to scientific excellence and its easy relations with scientists from a wide range of developing countries, should take a formal and special interest in ensuring that such scientists are kept fully in touch with developments in prediction and the assessment of seismic risk. ICTP already takes a similar special interest in solid state physics, a subject with relevance to the developing world.

This initiative should be widely welcomed. (The Editor declares an interest, having been involved in the discussions.) Of course there are international unions and associations which could also have performed this function to a degree, but satisfaction at the possible commitment of ICTP in this work will far outweigh any quibbles over who else could have done what.

In the longer run ICTP, in conjunction with UNESCO maybe, could possibly participate more actively in the raising of political consciousness on earthquake hazards. A model already exists in the team of examiners which occasionally emerges from OECD in Paris to visit a developed country and examine its science policy. A team of experts in earthquake protection could perform a similar role of persuading politicians to take their duties in hazard reduction seriously. □

The North American North (1)

# What strategy for development?

David Spurgeon in Ottawa summarises a Science Council report on the development of Canada's North

**L**ITTLE-KNOWN to the rest of the world, Canada is a country about which myth and misconception abound. Many people are especially ignorant of Canada's North, but that is scarcely to be wondered at, for so are Canadians themselves. And lately, many of the myths have been challenged from within, including the myth of self-sufficiency in energy and minerals. No less than the economic adviser to the Progressive Conservative party, James Gillies, has wondered about Canada as a technologically advanced nation and suggested that the country should return to reliance on selling its natural resources.

This orgy of self examination has also included some fundamental studies of the North, and the latest is a report from the Science Council of Canada. Called *Northward Looking: A Strategy and a Science Policy for Northern Development*, it is the result of 3½ years' preparation and sets out a strategy designed to bring more economic and technological self-sufficiency to the North, and a set of principles that should guide science policies for northern development.

But to set the stage, it tells Canadians a few facts about the part of their country—the largest part—that few of them have ever seen. The perspective bears repetition. Canada is the second largest country in the world, and the Yukon and the Northwest Territories together comprise 41% of its land area. As the report puts it:

Canada ranks 33rd among countries of the world in population. There is no settlement in 89% of our land area. In 1971, our average population density south of 60 degrees was 1,024 people per hundred square miles; for Nova Scotia, 3,867; for Ontario, 2,239. In the Yukon it was 9, and in the Northwest Territories, 3. Half of the people in the Northwest Territories and 60% of those in the Yukon live in *urban* environments. The northern part of Canada is mostly a lonesome place.

The North, says the report, is characterised by "small populations, a short growing season, permafrost, and long, cold, dark winters. The Extreme North and the Middle North are very different. The Extreme North is nearly uninhabited, has very little vegetation, continuous permafrost, ice-infested waters all or nearly all year, and very little precipitation. The Middle North has discontinuous permafrost, is heavily forested, more accessible, and is the

focus of resource exploration at this time. It is the region where the large majority of northern residents now live".

The people of the North, as distinctive as their environment, present problems for demographers. They move over large areas, there are cultural and linguistic differences, and many settlements are relatively isolated. Demographic statistics are thus more crude than elsewhere in the country. No accurate population figures exist on Métis and non-status Indians in Canada. The Native Council of Canada estimates 750,000 people on the basis of three Métis for each status Indian (295,215 in 1971 in all Canada). The total population of the North is about one million. Compared with the rest of Canada, they are younger and have received less formal education. There is a larger component of native people (especially in Saskatchewan, Manitoba, Quebec, and the Northwest Territories), and larger proportions of the population are engaged in forestry, fishing, trapping, and mining than in Canada as a whole.

Large communities are uncommon in the North: in 1971 only 29% lived in settlements of over 10,000, compared to 65% in Canada as a whole. Within the North, a community of more than 2,000 is an urban environment. Most of the immigrants settle in the larger centres: more than 70% of the non-natives live in the five largest communities of the Northwest Territories—Yellowknife, Inuvik, Hay River, Fort Smith and Frobisher Bay.

Epidemiological data indicate that health standards in the North are lower than in the rest of Canada. And although the birth rate has now begun to level off, the age structure of the native population resembles that of Latin America or Africa more than the rest of Canada. Twenty per cent of the inhabitants of the Northwest Territories speak neither English nor French, there are several native dialects, and in the Mackenzie River Valley and Delta, six native languages. "There is not really just one North in Canada", says the report. "The diversity of the North is as striking as its cold".

## Historical overview first

To study this area, the council undertook a historical overview and case

studies of northern development. The work was synthesised in a discussion paper, which then served as the basic document for seminars and to solicit opinion from more than 100 people involved in northern affairs. All this led to the framing of the final report. The chairman of the Science Council Committee on Northern Development, W. H. Gauvin, notes in an introduction that the process covered a period when many important issues were emerging: the OPEC oil embargo, land claims by natives in the James Bay area, the first drilling from artificial islands in the Beaufort Sea, preparations for northern pipelines and extraction of oil from the sands and so on.

It is against this background that the report's recommendations must be set. It notes that the recent history of the North has been the product of two conflicting trends: the thrust toward large-scale exploitation of natural resources, and the desire to continue traditional resource harvesting activities such as fishing, hunting and trapping, which would lead to development based on smaller scale, locally controlled projects.

"The Science Council believes that both major trends should be accommodated in a *strategy of mixed development*", says the report. "Such a strategy would press for more economic and technological self-sufficiency for the North. Activities that can be logically defined and controlled would be favoured over those which tend to increase political and economic dependence, the need for welfare, or other undesirable social conditions. This means an emphasis on relatively low capital, decentralised, and small scale development".

The objectives of northern development espoused by the Science Council are similar to those expressed by the federal government: to promote the welfare of northern people, to maintain and enhance the regenerative capacity of the environment, to give renewable resource development a higher priority, and to encourage economically viable non-renewable resource projects that are in the national interest and will benefit—or at least not harm—northern residents and their environment.

This strategy of mixed development will require greater sensitivity to traditional patterns of land use, says the report. It also says it will involve extracting mineral resources more slowly than some "major participants" may desire. Ironically, shortly after the report appeared, the Energy Minister, Alastair Gillespie, was proposing encouraging exploration by the multinational oil companies by offering them tax cuts. The report emphasises



that the land has special significance for many native northerners, and needs to be valued in terms of its capability to meet a variety of needs not measurable in monetary terms.

The report also calls for greater recognition of the value of public participation in projects concerning the North. "The right of people directly affected by a project to have something to say about it is becoming more widely accepted. . . . In the North, in fact, complaints about adequate *consultation* have been so frequent that the question of *participation* itself is only now being raised. To pursue successfully the strategy of mixed development, there must be more than informing, educating, and consulting northern people about the needs of the rest of Canada for a certain project".

The report lays down four principles that should guide policies for northern development:

- Technological sovereignty: the ability of Canadians to control, direct and benefit from technological enterprises deemed essential to the country.
- Life-style flexibility: the need to allow opportunities for choices of life style.
- Maintenance of the regenerative capacity of the land.
- Comprehensive and balanced assessment and monitoring of large and small projects.

These four principles, it says, should govern the choice of all new research and development initiatives in the North.

Technological sovereignty has become a buzz word at the Science Council. The reason for applying it to the North is that there, as elsewhere, "Canadians and Canadian science and industry tend to place undue emphasis on foreign expertise and foreign consultants". The report says that foreign-owned firms tend to perform the research that has the greatest potential for long-term payoffs in their home countries.

In offshore Labrador, for example, where several companies are searching for oil and developing the expertise necessary for that environment, Canadian firms have participated in the data collection phase, but they have had very little to do with planning and design of production and transportation facilities. The nature of research activity in Canadian resource-related industries, then, tends to resemble the resource extraction industries themselves. That is, with few exceptions, Canadians provide the raw materials, but the control of the operation, the processing and, hence, long-term benefits tend to flow out of the country.

In order to support the proposed strategies for northern development the Science Council makes a number of proposals. One is that universities should play a greater role in the solu-

tion of northern problems. In spite of the efforts of the universities of Alberta, Saskatchewan, McGill, Chicoutimi, Laval, Memorial (in Newfoundland) and others, most northern research is now performed by industry and government. Canada needs a cadre of researchers not dependent on contracts from interested parties, the report says. The Science Council urges that funds for northern research be re-allocated so that grants are emphasised over contracts, and that the granting councils provide funds for logistic support over and above other costs, because northern research is costly.

In addition, the Council urges the establishment of a University of the North to provide the focus for northern research. This university should concentrate on such areas as resource management and systematising resource inventories, and should promote the innovation of northern technologies. Native peoples should play a central role in the choice of

research topics and in undertaking research. Funding should be primarily from the federal government, and the university at first would give only graduate degrees.

The report emphasises the need for an inventory of renewable resources, and other data on the North, and the need for improved communications. There is also a need for coordination and exchange of the information that would come out of a northern data bank system, particularly among those who fund northern research. "It is intolerable that Members of Parliament should regularly report difficulties in gaining access to technical and scientific information relevant to political decision-making", it says.

In the past, most scientific exploration of Canada's North has been carried out by scientists of other countries, and much of the North's development has been for the benefit of others. If the Science Council's recommendations are acted upon, that era may end. □



*Bleak outpost at Hudson's Bay*



*Prospecting for metals in Canada's North*



The North American North (2)

## Money or energy problem?

Jeff Carruthers in Ottawa examines the most expensive private capital project ever undertaken, the AlCan gas pipeline

CANADA and the United States have together decided to build an all-new gas transmission system to carry Alaskan natural gas more than 5,000 miles to markets in North America's industrial heartlands in the North and West. The planned pipeline can be described in many ways, all of them large. The cost alone will be at least \$10,000 million. Ultimately the system might also transport smaller gas reserves from Canada's nearby petroleum fields in the Mackenzie Delta and Beaufort Sea region of the western Canadian Arctic, to its own consumers in central and eastern Canada.

From a financial perspective, the Alaska Highway pipeline project is almost certainly the most expensive private capital project ever undertaken. Economists and bankers generally agree that financing could stretch Canadian and American money markets to their limit, especially if the inflation and cost over-runs that have plagued other recent North American energy projects (such as the trans-Alaska oil pipeline and the mammoth James Bay hydroelectric project) push costs for the Alaska Highway project to the \$15,000 million or \$20,000 million that some experts fear.

From a technical viewpoint, the problems to be encountered in the northernmost areas in Alaska and the Canadian Yukon Territory are considerable, but at the same time not regarded as insoluble. The key issue is whether they can be resolved within the time and cost parameters agreed to by the two governments. A frigid and dark climate prevails during the winter construction months. Gravel and water are difficult to find in places for construction. The permafrost, where materials hard as rock in below-zero temperatures can turn to soup if thawed, and where a phenomenon called 'frost heave' can literally lift a pipeline out of the ground if it is intentionally chilled through water-rich permafrost soils, presents its own problems. And an ecology that in many places is poorly understood needs protecting.

Economically speaking, Canada regards the pipeline project almost as a godsend, calculating that it could provide upwards of 100,000 man-years of direct and indirect employment for an economy facing rising unemployment, a worsening balance of trade and a damaging rate of inflation. In fact the

project is widely regarded as much as a make-work scheme as a vital (if costly) way of developing urgently-needed energy supplies.

### Energy initiative

Yet it is the energy initiative inherent in the pipeline project which puts the Alaska Highway cooperative venture into its proper perspective. For \$10,000 million the United States hopes to connect gas reserves of 22-24 trillion cubic feet (TCF) in Alaska's Prudhoe Bay oil fields to markets in the lower 48 states. Assuming all of that gas is available—and there is some controversy about this already, based on fears that extraction of the gas could lower the recovery of associated (and more vital) crude oil—it would be sufficient to fill one year of current US gas demand.

Canada, which is hoping the pipeline can be connected later to some 5.2 TCF of gas in the Mackenzie Delta, would be able to fill two years of its current demand with its northern gas from the Delta. Ironically, perhaps, Canada is slated to export more natural gas from already-connected fields in southern Canada to the United States during the next decade than it hopes to connect in the western Arctic—and this is gas costing considerably less than Alaskan and Delta gas (the best estimates at present are that the Alaska Highway pipeline will deliver northern gas to southern markets for between \$3 and \$4 a thousand cubic feet, assuming no major cost over-runs for the pipeline project).

Critics of the pipeline include church groups, energy conservation groups, and environmentalists and northern native groups. They have argued that Canada and the United States do not really need the extra gas and that the adoption of adequate energy conservation techniques nationally could probably do more good for the state of the two nations' energy than any mammoth project. They have managed to focus national attention on a stark reality: that replacement energy for wasted existing energy resources in Canada is costing so much to develop on a per unit basis that even supposedly resource-rich countries like Canada cannot continue to rely on the non-renewable fossil fuels indefinitely.

If anything, the Alaska Highway pipeline underlines the growing belief that the availability of money is rapidly becoming more critical than the avail-



Building the trans-Alaska oil pipeline

ability of new energy resources, especially as the new resources grow more and more remote either geographically, as with Arctic gas and oil, or technologically, as with Canada's vast oil sands deposits.

### Two competitors

This relatively new concern about finance, which ultimately translates into energy costs to consumers, was one of the key factors in the two governments' selection of the Alaska Highway project over two competing gas transmission projects.

One rival consortium had proposed a pipeline from the oil and gas fields across the environmentally-sensitive North Slope (with the Alaska Wildlife range) to the Mackenzie River Delta, then up the Mackenzie River valley in the Northwest Territories to the province of Alberta, and then into the United States and central Canada. This proposal, by Canadian Arctic Gas Pipelines Ltd, a US-dominated consortium, was rejected in Canada on socio-economic and environmental grounds, even though it was believed to be technically superior to the Alaska Highway project. Approval of the project would have meant opening up the relatively lush Mackenzie Valley and would have risked confrontation with militant native groups, which are still trying to settle aboriginal claims with the Canadian government for much of the area.

The Arctic Gas project, as it was called, was the front-runner until a few months before the final government decision. A historic northern inquiry by a provincial Supreme Court judge, Thomas Berger, had been launched by the government to study the impact of such a development on the North generally and on the Mackenzie Valley specifically. Berger's report dealt a death blow to the Arctic Gas project when it suggested that the native way



of life for northern Eskimos and Indians would be ruined should the pipeline proceed within the decade. Berger looked more favourably on the Alaska Highway project, in part because a highway to Alaska had already disrupted that part of the North and because the natives were already more integrated into a southern style of life.

Other factors helped to kill the Arctic Gas project. The small Canadian gas reserves in the western Arctic, for example, did not justify an immediate Canadian commitment to a joint pipeline with the United States (the Alaska Highway project, by contrast is intended initially to serve only the United States). Arctic Gas also said it needed government guarantees to be able to finance its slightly-larger project—guarantees neither government was willing to provide. In addition, 'frost heave' and winter construction problems were more severe along the Mackenzie Valley route (the consortium would have had to provide 24-hour-a-day artificial lighting and artificial snow to construct the most northerly portions). Finally, the Arctic Gas project probably wouldn't have been Canadian controlled.

In the last analysis, the Arctic Gas project was an engineering and technical solution, based also on geological prospects along the route. But it failed to be sufficiently flexible to cope with non-technical matters, including its impact on native northerners, on Caribou herds, and on the ecology of a major river valley and a delta in the Arctic—not to mention political matters such as Canadian nationalism. To add further to the irony, the Arctic Gas proposal was studied to a much greater extent than the Alaska Highway project, so that more of the problems associated with it were known and widely publicised by its opponents in the media and before regulatory

review bodies.

Another petroleum industry consortium, preparing officially to propose the construction of an even longer and more technically difficult gas pipeline from the High Arctic of Canada (Melville Island initially) to southern markets, is keenly aware of this danger of being too prepared. The Polar Gas consortium, as it is called, is proposing a step-by-step government review in which the broad economic, social and environmental issues are dealt with first, preferably to produce some sort of initial government approval. Then more studies would be done and more money spent on the technical aspects of getting a 42-inch gas pipeline safely to the mainland, across marine trenches tens of miles wide and as much as 1,500 feet deep. The Polar Gas project is expected to cost even more than the Alaska Highway project, and to tap 10 to 15 TCF of gas for Canadian and possibly American markets.

### Second project

The other project defeated by the Alaska Highway pipeline was the El Paso scheme to transport the Alaskan gas in liquified form by tankers to the west coast. The US government decided that a pipeline across Canada was more economical and involved less risk because a well-proven technology was being used. Since that decision earlier this year a number of Canadian companies (including PetroCanada, the national petroleum company, and Alberta Gas Trunk Line Company Ltd of Calgary, one of the two founders of the Alaska Highway pipeline consortium in Canada) have been studying the feasibility of using special ice-strengthened liquified natural gas tankers to transport High Arctic Islands gas from Melville Island to markets along the eastern seaboard. The project, if it is ever pursued, will initially move only 250,000 cubic feet a day, compared to more than 2,000 million cubic feet a day to be moved along the Alaska Highway pipeline starting in 1983.

In allowing the Alaska Highway pipeline to cross Canada, the Canadian government has insisted that the Canadian portions be Canadian controlled and has accepted the promises by private industry (the Foothills Pipelines Ltd consortium) that Canadian content in the Canadian portions would exceed 90% for goods and services. The government is assuming that in a few years the go-ahead will also be given to a lateral pipeline to the Mackenzie Delta, and space has been reserved in the pipeline for this Canadian gas.

The US government has even agreed to have its gas consumers pay for a

substantial portion of the lateral pipeline to the Mackenzie Delta, on the condition that cost over-runs in the Canadian sections of the main pipeline are kept below 35%. As a further incentive to keep costs under control, and with the nightmare cost over-runs associated with the TransAlaska oil pipeline still fresh in mind, the two governments plan to tie approved return on equity for the private participants to the success in minimising costs.

The US Congress has recently given legislative approval for the project. Legislation should be introduced in the Canadian parliament before the end of the year which will also establish a single monitoring agency to ensure that environmental and native impact is minimised and to ensure that safety standards are met.

Finance is the next major hurdle, with \$8,100 million due to be raised in US capital markets and \$1,700 million in Canada. The first official attempts at financing should take place late next year. And should private financing in North America fail, the pipeline consortium is expected to seek government assistance in Washington and Ottawa before trying to tap European and other foreign money markets.

The pipeline project promises major business and major technological challenges for many key industries in Canada and the United States—steel, construction equipment, valves and turbine compressors, ditchers and welding machines, engineering and consulting services. It should therefore come as no surprise that the Canadian government hopes to use the Alaska Highway project as a lever for convincing more foreign companies to start working and manufacturing in Canada, using more Canadian talent instead of just Canadian raw resources.

On the planning boards in government offices, the Alaska Highway project is only one of a long list of expensive and increasingly technically difficult energy projects, including tidal power, oil sands extraction plants, heavy oil extraction and up-grading plants, more northern gas pipelines, LNG tanker transportation schemes, nuclear power (using the CANDU system developed in Canada), hydroelectric developments (including the use of DC transmission for long-distance power movement), offshore exploration, and coal gasification plants.

As with the Alaska Highway pipeline, the uncertainty surrounding these projects relates more to the availability of financing than of technical expertise. And the Canadian government says it is willing to invest in equity in such projects if necessary, if only to make sure the money is available. □





## SPACE

# Meteosat's turn

*Judy Redfearn reports on Europe's latest satellite*

METEOSAT, Europe's first satellite devoted entirely to watching the weather, was scheduled for launch this week from Cape Canaveral into a geostationary orbit 35,900 km above the equator. But a valve in the second stage of its Thor Delta 2914 launcher developed a leak last weekend. NASA has announced that the launch will be delayed, until it can replace either the launcher or the valve.

When Meteosat finally arrives in its planned orbit it will take images of the earth's surface and cloud cover both in the visible and infrared which will be used by European meteorological services to improve their long range weather forecasting. The idea to build a geostationary meteorological satellite was included in the European Space Agency's (ESA) optional application programme in 1971.

Meteosat will, in fact, be only the

first of several European meteorological satellites. The second is already approved and should be ready for launch in the early 1980s and subsequent ones are under consideration. Meteosat therefore has a dual role: it is partly pre-operational, designed to prove a satellite system, but it is also an important scientific satellite in its own right.

From its position in space, Meteosat will relay back to earth meteorological data on most of Europe, the whole of Africa and the Middle East. Together with four other geostationary satellites positioned symmetrically above the equator, two American (Goes), one Soviet (GOMS), and one Japanese (GMS), it will make weather monitoring of the whole globe possible between latitudes  $+50^\circ$  and  $-50^\circ$ . The five satellites will be taking part in the first global experiment, running from the end of 1978 to the end of 1979, of the Global Atmospheric Research Programme (GARP).

The Japanese GMS was launched last July, and the USA already has

three Goes satellites up—two it will use and the third it is storing in space as a spare. But the Soviet Union's satellite, due for launch in about a year's time, is unlikely to be ready because of technical difficulties. NASA's precaution in keeping a stand-by ready may yet save the first global experiment, though no decision on how to replace the Soviet satellite has been taken.

A feature distinguishing Meteosat from other satellites is the way in which the data it generates is processed. Because of the enormous data volume—two images every half hour—all processing must be done in real time. The facility set up especially to do this and to disseminate information to the users is at the European Space Operations Centre (ESOC) at Darmstadt. After the images have been corrected for distortion at Darmstadt, they are relayed back to the satellite and then on to the users.

Should Meteosat itself fail, however, there will be another chance. As with the Orbital Test Satellite, ESA took out insurance for \$16 million to cover costs of a replacement launcher and integration of a second satellite. □

## SWEDEN

# Fälldin's energy puzzle

*Wendy Barnaby, in Stockholm, updates nuclear developments in Sweden*

CONSERVATION groups in Sweden recently succeeded in stopping a long-disputed application to mine the bulk of Europe's uranium. The application, which the mining company LKAB had previously withdrawn and re-presented taking more account of environmental considerations, was to mine 200 tons of uranium a year for ten years from an area about 350 km south-west of Stockholm, where the uranium deposits are estimated to be the largest in the West in the price range of \$10–15 a pound (1968 prices). The local government body used its right to veto any project involving such widespread damage to the environment which, it maintains, is rich in cultural treasures. A spokesman for the company said that it would now begin discussions with the government and that this could lead to a research and development project going ahead, with a small amount of mining to sustain it.

But the action taken over the LKAB application is only one of the pieces, a relatively small piece, of the country's

energy puzzle. There are many others that have yet to be fitted in. At the centre of the government's difficulties is the interpretation of the law which specifies the conditions under which the building of reactors may continue. Under the law, the owners of any reactor being planned or under construction must present the government with concrete proposals of the 'completely safe' storage of unprocessed waste or of highly-radioactive waste, if the spent fuel is to be reprocessed. Reprocessing agreements must also have been concluded before the government gives permission for the reactor to be built.

Although the owners of Barsebäck 2 and Ringhals 1 and 2 reactors have concluded agreements with the French Cogema company for reprocessing spent fuel, there is some doubt that the government will recognise the agreements as fulfilling the conditions of the law. This is because of a report prepared jointly by a committee of Cogema's trade unions, employers and safety representatives, which demanded that security at the plant be improved to meet national and international standards and itemised 47 points on which improvements in security and operations in general could be made.

Critics here say that the spirit of the Swedish law demands reprocessing agreements which will guarantee workable, reliable reprocessing. The reactor owners maintain that their responsibility under the law stops with the signing of legal agreements, and that the security matters to be taken care of at the plant are Cogema's business, not theirs. They are happy, they say, to trust the Frenchmen's technical abilities to solve the problems at their end. Overshadowing the entire deal is a query about the American government's attitude. There is no guarantee that the Americans, who supply Sweden with its enriched uranium and can veto the export of spent fuel from Sweden for reprocessing, will allow it to go ahead.

The government must soon decide which interpretation of the law it is to favour. It has given one reprocessing agreement to the state Nuclear Power Inspectorate for comments, and these are expected to be published late in November. The government's decision is expected in December.

December will also see the first of two 'security reports' being presented to the government by the nuclear power industry. The first one will deal with storage of reprocessed spent fuel, and will maintain that safe storage is technically possible. The second, to

*(continued overleaf, page 202)*



## PROFILE

Chris Sherwell on Sir Hermann Bondi

## An effective use of oil

HERMANN BONDI followed an auspicious precedent in British government when he left the Ministry of Defence to take over as chief scientist at the Department of Energy seven weeks ago. Before him, only Solly Zuckerman and Alan Cottrell had ever held the position of chief scientist in two separate departments of state—though they moved from Defence to the Cabinet Office, and in the days before the Rothschild reorganisation gave the institution its present form.

The appointment itself, when it finally came, was no surprise to anyone who had bothered to read the newspapers. But there was a gap before it was announced while the minor storm generated by the departure of Bondi's predecessor, Walter Marshall, subsided. The gap which Bondi himself left at Defence remains, and though names are being canvassed, no indication of who is in the running is officially available.

Bondi came to Energy with a remarkable record. Schooled in Vienna, where he was born 58 years ago, he went to Trinity College, Cambridge, and then became what was bureaucratically called a 'temporary experimental officer' in the Admiralty from 1942–45. In 1954, after holding a Trinity fellowship and lecturing for the best part of eight years, he was appointed to the chair of mathematics at King's College, London. Then in 1967 a leave of absence allowed him to become director general of the European Space Research Organisation. He stayed until 1971, when he became chief scientist at the Ministry of Defence.

The experience at Defence colours his approach in his new post. He regards the issue of nuclear proliferation as particularly important, for example. But as he puts it, "Things have to be looked at with a certain order of priority". Short term problems like proliferation must be checked before long term ones can be tackled. If the world is going to blow itself up first, he is not too worried about, say, the threat of a 'greenhouse effect' from over-use of fossil fuel.

Not that he has no views on the greenhouse effect. He says the US National Academy of Sciences report published earlier this year has been read as "more definite" than he reads it. If atmospheric oxygen comes from living plants making use of carbon

dioxide, he asks, and rising carbon dioxide levels can encourage plants to grow and so stabilise the rise, might not preservation of the most active plants in the best parts of the world be better than a restriction on the use of coal? It is a question for the long term, Bondi says simply, which will be of no interest at all unless we attempt to ensure our survival in the short term.

He is reticent about giving his own views on such immediate issues as maintaining the breeder option or Britain's choice of reactor. He stresses that energy as a whole, and not just nuclear power, is capital intensive and expensive, the turnover of the energy industries being about the same as the defence and education budgets combined. As he sees it, R&D in alternative energy technologies must be fostered in case conventional sources become unacceptable, unavailable or too expensive, but the judgment of likely fuel costs is complex.

One economic consideration which puzzles him is the effect of the oil price increase four years ago. The developed countries initially responded by saying they would maintain their economic growth and replace oil with nuclear power. That led to colossal predictions for the growth of nuclear power, says Bondi. "But the calculations were completely bogus". The fuel crisis stopped growth, and nuclear forecasts slumped. Instead of energy prices rising relative to other things, inflation and a lack of growth caused fuel costs in many fields to become a smaller share of the total than they were. "So if I'm asked whether a project for an alternative energy source would become economic if the real price of fuel doubled, I must ask if engineering costs will go up equally disproportionately, and whether we'll be back where we started".

As Bondi sees it, a rise in fuel costs, rather like a failed harvest in developing countries, has to mean a substantial drop in the standard of living. But developed countries have been totally unwilling to accept this. Is there an appreciation of this within government? "I preach it", Bondi replies with a smile. He is as aware as anyone of the problems governments face, as he shows when he allows his thoughts about costs to stray to current issues. The difficulty



Sir Hermann Bondi

when it comes to choosing a reactor or investing in alternative energy sources, he says, is not really a lack of money, but spending it sensibly and responsibly—not whether to purchase, but what to purchase.

So how should a chief scientist be seen? "Like any scientist", Bondi says, "the chief scientist has a particular standing when he gives a judgment on scientific matters. But he wouldn't be a responsible citizen if he didn't have a judgment on other matters as well, although he has no more claim to authority on these than anybody else". Bondi regards the important element in the chief scientist's task as "making clear what the scientific issues are, within the context of other questions".

He is clear why he came to Energy. First of all he was asked, and he has an appetite for change. Another attraction was that it was controversial. But there is a third aspect, which has to do with his time at Defence. He enjoyed his time there, he says, and they enjoyed having him. He rejects completely any suggestion that they wanted to see him go. But the contribution of somebody from outside springs from the person's different background and different way of thinking. "He treads water for a while, learns to understand the issues and how to handle the machine. Then he can make a distinct contribution. But after some years he loses the advantages an outside background is supposed to confer".

He has now applied the lesson to himself. At Defence, he says, he had become part of the furniture. "I was with my fourth secretary of state, my third permanent secretary, and my sixth chief of defence staff". Continuity has its own contribution to make, he says. "But I'm not convinced that I was bringing to it as much as I had done, say, two years ago, though"—and this with a twinkle—"I knew very well how to put a drop of oil into the machine". He now has more oil, and a smaller machine.



come next spring, will deal with storage of unprocessed waste, and will no doubt be optimistic too.

election is, according to Fálldin, a distinct possibility.

With Fällidin's position hardening, the government will have to deal with applications expected from the State Power Board early next year to load the Ringhals 3 and Forsmark 1 reactors. The Prime Minister has already refused to allow Ringhals 3 to be loaded once, saying that the State Power Board had not fulfilled the security conditions, and he has recently said that if it is loaded he will resign. March will be no less momentous a month. President Carter is then expected to enlarge on his proposal to store Sweden's (and other countries')

unprocessed spent fuel in the USA. Fälldin's coalition partners are reportedly willing to accept Carter's invitation, but Fälldin is doubtful about releasing spent fuel to storage facilities which have had their own technical troubles. Also in March the Energy Commission is to present its report on alternative energy futures to Parliament. Whether it will be able to come up with some viable transition to environmentally-benign energy production is as yet unknown. But unless it does, Fälldin's tactics seem bound to end in either a devastating loss of face for his party, or in his own resignation. □

which the somatostatin could be chemically cut off.

How important, in general, is this technique? And specifically, how important is the artificial production of somatostatin? The main point

[illegible]

There is no doubt that this latest achievement, which is due to a team led by Herbert Boyer of the City of Hope Medical Center in California, is an important technical step forward. Insulin genes were successfully transplanted into bacteria earlier this year, but it has so far proved impossible to get the bacteria to produce insulin from them. Boyer's team, instead of isolating and transplanting a real gene, synthesised a nucleotide sequence corresponding to the sequence of 18 amino acids that go to make up somatotropin. That gave them an 'artificial gene' which they have not only inserted into bacteria, but persuaded the bacteria to decode into somatostatin.

Boyer and his colleagues have been reluctant to disclose details of their experiments because the work is still unpublished. But it seems that the 'artificial gene' was inserted into a plasmid containing part of the bacterial *lac* operon—a package of bacterial genes that controls the production of the enzyme  $\beta$ -galactosidase. The somatostatin gene was placed in the middle of the  $\beta$ -galactosidase gene, where the two genes would be transcribed together under the control of the *lac* promoter gene. The resultant RNA was apparently translated into a hybrid protein from

about the technique is that it shows that it is possible to induce bacteria to make animal gene products. The demonstration that this can be done with synthetic genes may have two important consequences. First, there is mounting evidence that the genes of higher animals are different from those of bacteria in that they contain regions of DNA that do not code for the final protein. Since it is unlikely that a bacterium would be equipped to cope with such discontinuous genes, it is important to know that a simple coding sequence can be used instead of an actual gene. Second, there are now many cases in which

what is needed for medical purposes is not the natural protein but a specific analogue of it. If bacteria can be made to produce the original from an artificial gene, then they can presumably also work on an artificial gene coding for an analogue.

There are also two important limitations on the technique, however. First, it is only practical to make synthetic genes for relatively small molecules, and small molecules are not very difficult to synthesise by traditional means. Whether genetic engineering will eventually prove the cheaper way it is hard to gauge. Second, the particular method Boyer's team used to clip the somatostatin out of the hybrid protein is not generally applicable. It depends on the fact that the bacterial protein contains a specific chemical group that is not present in somatostatin. However, it is present in most proteins.

Somatostatin itself is unlikely to be very widely used for clinical purposes. It inhibits the release of the hormones glucagon, thyrotrophin, prolactin, gastrin and insulin. Because of its inhibition of hormones that act against insulin, it has been clinically tried in cases of extreme insulin-resistant diabetes (less than 5% of diabetics). But because of its effects on other hormones, and the fact that it inhibits the aggregation of platelets and may thus predispose to haemorrhage, it is most unlikely to find more widespread application. Attempts have been made to find analogues with more specific action, but they have so far failed and it may be that all the diverse actions of the neurohormone depend on the same chemical feature.

At this stage, therefore, the remarkable achievement of Boyer and his collaborators remains an advance in principle rather than in practice.



## IN BRIEF

## Reactor choice soon

Consultations held by Britain's energy minister, Mr Anthony Wedgwood Benn, on the country's choice of thermal nuclear reactor, are drawing to a close now that most submissions from interested parties on the subject have been received. A decision is expected before Christmas.

The latest submission on the issue has come from the Trades Union Congress (TUC), representing Britain's labour movement. Its fuel and power industries committee wants the immediate ordering of an AGR station with another later, and a major effort to work up a detailed and acceptable PWR design before a decision on a third station is made. The committee also supports the construction of a demonstration breeder reactor and of expanded reprocessing facilities at Windscale.

Last month the country's two main electricity boards, the CEBG and SSEB, submitted their views to the Department of Energy. The department has also published virtually in full the report of an assessment of the three reactor types under consideration by the National Nuclear Corporation.

## ACORD members named

The chairman of the UK Science Research Council (SRC), Professor Geoffrey Allen, has stepped into another appointment recently vacated by his predecessor at SRC, Sir Sam Edwards: membership of the Advisory Council on Research and Development for fuel and power (ACORD). Dr Joseph Gibson, the board member for science at the National Coal Board, joins ACORD as NCB representative.

ACORD, with 10 longer-serving members drawn entirely from industry,

will shortly have to take a serious decision: how to advise Benn on the Severn Barrage schemes for tidal power, which will help to energise Britain but may radically alter the ecology of her greatest estuary.

## SA's nuclear optimism

With the controversy over South Africa's nuclear intentions still volatile, the president of the South African atomic energy board, Dr Ampie Roux, has said that if the United States cuts off supplies of nuclear fuel for the Koeberg nuclear station north of Cape Town, his country's uranium enrichment process could ensure supplies within a few years of the station's start-up date in the early 1980s. Dr Roux also said that South African uranium output would increase to make her the West's second largest producer after the United States.

SOCIOBIOLOGY seeks to explain that human social behaviour has a genetic, and, therefore, an evolutionary background, which has long been obvious for other orders of animals, especially the *Hymenoptera*. Against the acceptance of this concept is the fact that many people dislike being compared with monkeys and object even more to being told that they resemble insects.

I am becoming increasingly nervous about my behaviour being the result of millions of years of evolution, a conclusion I have to reach if I embrace the teachings of sociobiology. The thought puts me on my mettle; I should try not to let my genes down. My behaviour is a rather poor illustration of a progressive evolutionary process, but the sociobiologists say that social evolution may indeed be occasionally reversible. Other nagging questions assail me: should I obey my impulses because these promptings are the result of inherited patterns of mental processes? Or should I suppress these impulses in the biological interests of benefiting my group? Or should I try to shape up so as to be a survival machine for my genes, which, says Richard Dawkins, "swarm in huge colonies, safe inside gigantic lumbering robots, sealed off from the outside world . . . their preservation is the ultimate rationale of our existence . . ."?

In any case, the subject of sociobiology merits attention because Dick Lewontin dislikes it, and he is a man of strong convictions on important issues. Moreover, perhaps sociobiology could be an interesting chal-

lenge to the pan-selectionist school of evolutionists, who object to the proposal, made by the so-called 'neutralists', that amino acid replacements in homologous proteins of different

## On sociobiology



THOMAS H. JUKES

species are often changes that have no effect on the properties of the protein, but are incorporated by genetic drift. The neutral model also says that many mutations are deleterious and are rejected by natural selection, and a few become accepted by a species because they are advantageous.

The opposing view states that each amino acid in a protein must have a unique survival value in the phenotype of the organism. Would ethologists extend this general principle to

behavioural phenomena? Perhaps the idea of neutral hereditary changes could be extended to explain certain habits that have little to commend them, or it could even account for harmless quirks that have no particular bearing on our survival as a species. A leading sociobiologist reassuringly says that only about 10% to 15% of human social behaviour is genetically based, but I don't know what has led him to such a quantitative assessment. The same author says, "The hypothalamic-limbic complex automatically denies . . . logical reduction by replacing it with feelings of guilt and altruism". In other words, our emotions, which are part of our inheritance, prevent us from reaching conclusions based on logic. This is a facile way of disarming criticism, of course.

It seems to me that sociobiology aggravates its opponents by the ingenuity with which it produces explanations to make observations fit a theory. To a considerable extent, pan-selectionism has done the same by finding an adaptive advantage for each phenotypic trait. We are told that even the hair-pattern on our backs was selected by evolution in our ancestors to help shed the rain as they crouched on the branch of a tree during a tropical storm.

Perhaps those who disbelieve and spurn sociobiological teachings may be responding to a genetic trait. The struggle against pre-ordained fate could in itself have a survival value. This struggle may include the rejection of theories that tell us we are in the grip of determinism imposed by our genes.

# news and views

## Gametes of malaria parasites

from F. E. G. Cox

It has always been one of the major aims of parasitology to maintain parasites in culture and to minimise as far as possible the use of human subjects or laboratory animals. A year ago, the possibility of growing all stages of the human malaria parasite, *Plasmodium falciparum*, in culture was brought a step nearer when Trager and Jensen (*Science* **193**, 673; 1976) described the continuous cultivation of the blood-inhabiting asexual stages of this parasite in a simple medium. The next stage in the malaria life cycle is the development of gametocytes (the stages that infect mosquitoes) from the asexual stages in the blood and in this issue of *Nature* (page 240) Carter and Beach describe how this can be achieved *in vitro*. The early stages of *P. falciparum* that invade red cells can develop into either asexual stages, a process which takes about 18–24 h, or into gametocytes which takes about 10 days (Smalley *Nature* **264**, 271; 1976). It was the development of the asexual stages that Trager and Jensen achieved. Carter and Beach observed that in cultures of *P. falciparum* maintained in

Trager's medium, without the dilution that is usually carried out, obvious immature gametocytes were present after the expected 10 days. The problem was how to bring these stages to maturity. This was achieved by adding 50% human serum instead of the usual 10% to the cultures and after 8 days morphologically mature gametocytes were present. The next step was to confirm that these gametocytes were functionally mature by transforming them into gametes and this was done by suspending the gametocytes in foetal calf serum at pH 8.0 or human serum at pH 8.2. Trager's culture medium requires a pH of 7.3–7.4 for the growth of asexual stages so it is essential to change the pH in order to bring about these transformations.

The gametocytes and gametes produced by Carter and Beach look normal in every way and occur in similar proportions of male to female (1:3.5 compared with 1:4) to those in humans. There is no reason to doubt that they should be able to infect mosquitoes. This means that it should now be possible to complete the asexual and sexual cycles of *P. falciparum* without the need for a mammalian

host. It will next be necessary to grow the liver stages of the parasite *in vitro* but this should not present any insuperable difficulties. In the meantime, numerous experiments on the infectivity of *P. falciparum* to mosquitoes have become possible.

The question as to how long the gametocytes of *P. falciparum* are infective to mosquitos is an interesting one. Hawking, Wilson and Gammage (*Trans. R. Soc. trop. Med. Hyg.* **65**, 549; 1971) calculated that gametocytes were produced in a circadian pattern and that they would be infective for only a short period. Bray, McCrae and Smalley (*Int. J. Parasit.* **6**, 399; 1976) on the other hand, found no evidence for a circadian rhythm and Smalley and Sinden (*Parasitology* **74**, 1; 1977) showed that, in humans, gametocytes are infective for 2 weeks or more. The observations of Carter and Beach suggest that gametocytes can mature to gametes for several days at least and this supports the observations of Smalley and Sinden. The technique itself will be useful in resolving problems of this kind in future. It should also be possible to test the effects of drugs on gametocytes—a hitherto relatively unexplored field. □

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## Coevolution of *Calvaria* and the dodo

from Robert M. May

MUTUALISTIC associations, in which two species interact in such a way that each benefits from the presence of the other, are fascinating for several reasons. For the theoretician, they are increasingly shedding light on the interplay between the long sweep of evolution and the immediate dynamical effects of population interactions (for example, King, Gallaher & Levin *J. theor. Biol.* **53**, 263; 1975). For the naturalist, and even for the pure aesthete, they provide some of nature's

richest wonders: the homes and food that many tropical acacias supply for their protective ants; the nectar supplied by flowering plants as the price for pollination; the almost erotic coupling between many tropical orchids and their obligate bee pollinators.

One class of mutualistic interactions we should all be grateful for is that between fruit-producing plants and the animals that disperse their seeds. The fleshy part of the fruit is the cost the plant pays to induce animals (typically birds) to eat the fruit; the associated benefit is that the seeds, having taken some time to pass through the animals,

are spread widely. The fruits we eat are the direct or horticulturally-improved outcome of such coevolutionary cost-benefit calculations.

In systems of this kind, a major design problem is producing a seed that can survive the passage through the digestive tract of the fruit-eating animal. A partial answer is to speed the passage of the seed; hence the mildly laxative effect of many fruits (notably prunes).

All this brings us, rather surprisingly, to the dodo. This large, flightless bird was endemic to the 700-square-mile island of Mauritius. Its disappearance by 1681 is probably the most familiar

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The Dodo (*Raphus cucullatus*)

example of an extinction directly attributable to man: 'The Dodo used to walk around/And take the sun and air./The sun warms his native ground—/The Dodo is not there!/The voice which used to squawk and squeak/Is now forever dumb—/Yet may you see his bones and beak/All in the Mu-se-um.' (Belloc, *The Bad Child's Book of Beasts*, Dover reprint, 1961; see also Hachisuka, *The Dodo and Kindred Birds*, Witherby, 1953). Stanley Temple (*Science* **197**, 885; 1977) has drawn attention to the fact that the large monoecious tree *Calvaria major*, which according to historical records was once abundant and exploited for lumber on Mauritius, also seems to have undergone a trauma dating back to the dodo's demise. There were by 1973 only 13 *Calvaria major* trees surviving in the remnant native forests of the island, and all 13 were estimated to be more than 300 years old. No younger specimens are known to exist, even though the surviving trees produce well-formed, apparently fertile seeds each year. None of these seeds now germinate naturally, even if planted in nursery conditions.

Temple notes that, although there are several examples where the elimination of a plant species has been deleterious for an associated animal species, the *Calvaria*-dodo system may be the first documented instance where disappearance of the animal partner has doomed a mutualistic plant species. The mechanism suggested by Temple is that the seed inside the *Calvaria* fruit developed a coat (endocarp) thick and strong enough to resist crushing in the powerful gizzard of the dodo. As a result, 'these specialised, thick-walled pits could withstand ingestion by dodos, but the seeds within were unable to germinate' without the coat first being battered and abraded by the stones in the dodo's gizzard.

In support of this hypothesis, Temple observes that the *Calvaria* fruits are today consumed by some frugivorous animals, notably a parakeet and a flying fox, but that none is large

enough to eat the fruit whole, and thus disperse the seed. The dodo was large enough, and therefore it was presumably the customer for whom the tree designed its fruit. There is fossil evidence that dodos ate *Calvaria* fruit. Furthermore, measurements of the crushing forces generated by the stones in the gizzards of granivorous birds from several taxonomic groups (ranging in body weight from 0.8 to 3.2 kg) give a formula which, when applied to the 12-kg dodo, suggest crushing forces of around  $10^4 \text{ kg m}^{-2}$  in its gizzard. *Calvaria* pits are strong enough to withstand these forces, but only just: Temple suggests that once the pit had been sufficiently abraded to reduce its size by 30%, the seed would be crushed. But by then some of the seeds would have passed through the dodo, albeit after a battering which would weaken the endocarp and facilitate germination of the infant plant within.

As an experimental test of his ideas, Temple force-fed *Calvaria* seeds to turkeys, whose gizzards generate crushing forces around  $4,000 \text{ kg m}^{-2}$ , fairly close to those inferred for dodos. Of 17 seeds, seven were crushed. The remaining 10 were either regurgitated or defaecated, some after up to 6 days. These 10 seeds were planted in nursery conditions, and three subsequently germinated. Temple concludes that 'these may well have been the first *Calvaria* seeds to germinate in more than 300 years.' □

## More evidence for a closed Universe from QSOs

from T. Kiang

FURTHER evidence that quasars (QSOs) can provide information on the nature of the Universe comes from the work of Chinese astronomers. Their work complements the recent observation of the ultraviolet spectrum of the QSO 3C273 by Davidsen *et al.* (*Nature*, **269**, 203, 1977; *News and Views* **269**, 195; 1977). The Chinese astronomers have constructed Hubble diagrams for various subsets of radio QSOs using a different luminosity indicator from Davidsen *et al.*, but come to essentially the same conclusion.

It has long been realised that one of the best means to decide whether the Universe is open (the declaration para-

meter  $q_0$  less than  $\frac{1}{2}$  and the Universe expanding for ever) or closed ( $q_0$  greater than  $\frac{1}{2}$  and the Universe eventually falling back on itself) is by means of the Hubble diagram, in which the redshift  $z$  is plotted against the apparent magnitude  $m$ . (The redshift is the reddening of the light from stars and galaxies due to their recession). But the relations predicted by different cosmologies become distinguishable only when the redshifts extend beyond the present limit ( $\sim 0.5$ ) for the galaxies. That is why the discovery of QSOs with much higher redshifts brought high hopes. However, these hopes were soon dashed, for the first Hubble diagrams for QSOs looked like pure scatter diagrams, showing hardly any trend whatever. Subsequently, some astronomers took the view that the QSO redshifts are 'intrinsic' and not 'cosmological' at all.

Most astronomers, however, held to the view that these redshifts are at least largely a distance effect, and that the featureless Hubble diagram is due to the sample being a mixture of many different types of object. In support of this view, Setti and Woltjer (*Astrophys. J. Lett.* **181**, L61; 1973) showed that, if the sample is restricted to QSOs with steep radio spectra, then the scatter is much reduced, and a trend of the expected kind can be seen. Similarly, Stannard (*Nature* **236**, 295; 1973) found that the same thing happens if only objects with flat spectra are considered. But in these diagrams, there is still a large dispersion, which is attributed to spread in luminosity within each type. Further progress thus awaits the identification of some 'luminosity indicator', and that is where J. A. Baldwin's recent demonstration of the correlation between  $L\alpha$  intensity and continuum luminosity comes in (*Astrophys. J.* **214**, 679; 1977). Using the equivalent width of  $L\alpha$  as a luminosity indicator, Davidson *et al.* identified five quasars with  $z$  between 2.3 and 3.4 which are presumably as luminous as 3C273 at  $z = 0.158$ . Here at last is a Hubble diagram for objects of a limited luminosity range, and this fact is probably more significant than the particular result they obtained ( $q_0 \sim +1$ ) which the authors are quick to point out, critically depends on what one assumes for the galactic reddening in the direction of 3C273.

The diagram by Davidsen *et al.* is not the first one of its kind. In a paper in the Chinese journal *Acta astronomica sinica* (**17**, No. 2, 134; 1977; English translation in *Chinese Astronomy*, **1**, No. 2, in the press) an entirely different luminosity indicator was used and Hubble diagrams for

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various subsets of radio QSOs constructed, leading to a value of  $q_0 = +1.38$ . The authors of this paper are Fang Li-zhi, Zhou You-yuan, Cheng Fu-zhen and Chu Yao-quan from the University of Science and Technology of China—names quite unknown in the West.

Confining their attention to those QSOs with resolved radio components, these authors propose that the linear separation  $D$  between the components be used as a luminosity indicator. (They eventually back up this suggestion by actually deriving a value for the rate of change of the optical luminosity with  $D$ ). Since  $D$  is not directly observable, a rather subtle statistical argument was used in getting plausible estimates of  $D$ . Starting with observed angular separation  $\theta$  and redshift  $z$ , and assuming a distance-redshift relation  $r = r(z)$ , one calculates the projected distance  $r\theta$ , which differs from  $D$  by a projection factor  $\sin \psi$ , say. Now, in an isotropic distribution of directions in space,  $\sin \psi$  is concentrated near its large end value of 1. The authors argue that what one can do here is to take only those quasars with  $r\theta$  values at or close to the observed maximum  $r\theta$  value at the given  $z$ , and then simply set  $D = r\theta$  for such objects. In this way, from a sample of some 90 radio quasars with known  $\theta$  and  $z$ , they picked out 26 for which such indicative values of  $D$  can be assigned. Separate Hubble diagrams were then constructed for various ranges in  $D$ .

Rather tight correlations consistently appear in these diagrams, which typically have 10 data points and a scatter of about 0.6 magnitudes. But the linear regression coefficient of  $m$  upon  $\log z$  departs significantly from 5, which means that the classical Hubble relation  $r(z) \propto z$  is no longer valid. In a self-consistent series of calculations, the authors then found that, in order to recover a slope of 5, the regression analysis must be made between  $m$  and  $\log(z - 0.19z^2)$ . This is equivalent to saying that the correct distance-redshift relation is of the form  $r(z) \propto z - 0.19z^2$ , and that is how the formally precise value of  $q_0 = +1.38$  was obtained, because in relativistic models, the coefficient of  $z^2$  here is equal to  $\frac{1}{2}(1 - q_0)$ .

Although the true uncertainty in this determination must be very much larger than the formal value implies, it is probably not as large as in the derivation by Davidsen *et al.*, because the sample is larger and the sample points more evenly distributed. The two results complement each other in that one deals with flat-spectrum objects and the other, mainly steep spectrum objects. They agree in saying

that the Universe is probably closed.

On the other hand, because of consistent failure in detecting sufficient mass in one form or other, astronomers in recent years have become more and more disposed towards the opposite alternative of an open Universe—this, for example, was very much the general feeling in the recent Symposium on Large-Scale Structure of the Universe, held at Tallinn, Estonia.

The determination of  $q_0$  tacitly assumes that no change of luminosity has taken place in time. Hence the challenge now is to find a physically plausible model of luminosity evolution that will account for both a low mass density and a high value of  $q_0$ .

The evaluation of  $q_0$  is only one of several results obtained by Fang Li-zhi *et al.* Many astrophysicists may find their result that the optical luminosity of a quasar decreases by 2.3 magnitudes for every megaparsec increase in the linear separation between its radio components to be even more important.  $\square$

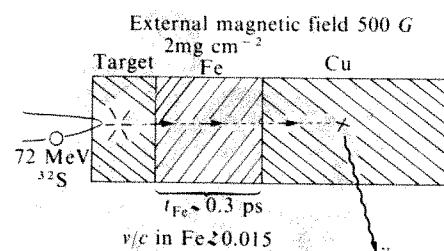
## Magnetic moments of short-lived nuclear states

from P. E. Hodgson

A NEW method of measuring the magnetic moments of nuclear states of very short lifetime has recently been developed by N. Benczer-Koller and colleagues at Rutgers University, and some preliminary results have just been reported at the recent conference on nuclear structure held in Tokyo in September. They have applied the method to several isotopes of iron, nickel and zinc and obtain results that are important for testing nuclear models.

In this method, a 72 MeV beam of  $^{32}\text{S}$  ions hits the target material, which is deposited on a  $2 \text{ mg cm}^{-2}$  layer of magnetised iron backed by  $10 \text{ mg cm}^{-2}$  copper, as shown in the figure. Some of the target nuclei are excited, and these recoil through the iron and stop in the copper, where they decay by gamma emission. The energy of the gamma ray identifies the state of excitation of the recoiling target nucleus.

The gamma rays are detected in coincidence with the  $^{32}\text{S}$  ions recoiling in the direction opposite to that of the incident beam. This ensures that the knocked-on target nuclei pass perpendicularly through the magnetised iron so that the length of their path in that



material is accurately known. As the target nuclei pass through the magnetised iron their magnetic moments precess and the angle of precession is measured by the rotation of the angular distribution of the de-excitation gamma rays,  $\Delta \theta \sim g \int B dt$ . The value of  $\int B dt$  and the constant of proportionality are determined by a subsidiary experiment using ions of known magnetic moment, so that a measurement of  $\Delta \theta$  enables  $g$  to be found. It is sufficient for practical purposes to use an empirical relation describing the dependence of  $B$  on the velocity of the ion; this is quite different from the theoretical relation, but this lack of understanding of the process does not affect the determination of the magnetic moments.

As an example of the results obtained, the magnetic moment of the lowest  $2^+$  state of  $^{54}\text{Fe}$ , that has a mean life of only 1.4 ps, is found to be  $1.68 \pm 0.38$ . Magnetic moments have also been determined for the lowest  $2^+$  state of  $^{58}\text{Fe}$ , and for the same states of four isotopes of nickel and four isotopes of zinc. In some cases the magnetic moments were already known from other work, and the new and old values are in good agreement.

This method has many advantages over those used previously. The high magnetic field inside a ferromagnet enables the magnetic moments of states with lifetimes in the ps region to be measured, whereas the methods using laboratory magnetic fields are limited to states with lifetimes in the ns region. Since the ion is moving rapidly through the magnetised iron and does not stop there the results do not depend on the rate of energy loss at low velocities. This is a major source of uncertainty in the methods in which the ions come to rest in the iron. Furthermore,  $\Delta \theta$  is independent of the lifetime of the ion providing it is sufficient for the ion to pass through the magnetised region.

The magnetic moments of excited states can be calculated from the shell model, and provide a searching test of its validity. The new results for the nickel isotopes disagree with shell model calculations that take no account of core excitation, but are in good accord with newer calculations that include  $f_{7/2}$  core excitation.  $\square$

P. E. Hodgson is a lecturer in Nuclear Physics in the University of Oxford.



## Epiphyte or parasite?

from Peter D. Moore

It is not difficult to define the difference between an epiphyte and a parasite; the former depends on another organism simply for support whereas the latter exploits its host in a manner which is normally beneficial to the parasite but detrimental to the host. Neat definitions, however, are not always easy to apply in complex field situations. Take, for example, the relationship between the robust, brown, fucoid alga *Ascophyllum nodosum*, which is abundant in the intertidal zone of most of the more sheltered shores around the British Isles, and the small red alga, *Polysiphonia lanosa*, which is almost invariably associated with it. The red alga adheres strongly to its host, since its thallus actually penetrates the tissues of *Ascophyllum*. Where the biomass of the host is greatest, there one finds the greatest density of *Polysiphonia* also. Here is a perfect situation in which the epiphyte/parasite debate can be staged.

One can see certain advantages in the epiphytic habit in this type of algal community. Elevation during immersion provides obvious benefits, for the mass of floating fronds of the brown alga, buoyed up by air bladders, must cast a considerable degree of shade upon any bottom-dwelling algae. At the same time, close association with *Ascophyllum* could make life more comfortable during the periods of immersion when extremes of variation in temperature, relative humidity, salinity and light intensity would be dampened by the presence and protection of a mucilaginous mass of limp fronds. Simple epiphytism would not, therefore, be an unreasonable expectation in such a circumstance.

The possibility that *Polysiphonia* is more than an innocuous passenger demands examination, however, for the attachment of the red algal thallus to its host seems remarkably secure for a species association which is found only where wave action is very slight. An examination of the anatomy of the attachment region by Rawlence and Taylor (*Can. J. Bot.* **48**, 607; 1970) showed that rhizoids of the 'epiphyte' penetrated deeply into the thallus of *Ascophyllum*.

Citharel (*C. r. Séanc. hebdom. Acad. Sci. Paris, Ser. D*, **274**, 1094; 1972) used a physiological approach to the problem, considering that, since no true epiphyte would take up metabolites

from its host, any demonstration of transport of reduced carbon from the brown alga to the red would provide grounds for an accusation of hemiparasitism. Citharel chose glutamic acid as the most likely vehicle of carbon transport, since this is the most abundant free amino acid in the *Ascophyllum* thallus. He injected  $^{14}\text{C}$  labelled glutamic acid into the thallus of *Ascophyllum* and observed its accumulation in *Polysiphonia* within 12 h. He concluded that, contrary to the opinion of such algal authorities as Fritch, *Polysiphonia* should be considered a partial parasite. The demonstration of such a transfer does not, however, necessarily infer that the red alga is dependent upon *Ascophyllum* as a carbon source, indeed Harlin and Craigie have argued that it is not (*J. Phycol.* **11**, 109; 1975).

A further complication has now been brought to light by translocation experiments by Turner and Evans (*New Phytol.* **79**, 363; 1977). They supplied  $^{14}\text{C}$ -labelled bicarbonate ions to *Ascophyllum* tissues and studied translocation of the label to parts of the plant isolated from the initial supply, and also into tissues of *Polysiphonia* attached to the host plant. In this way they hoped to overcome two possible objections to the experiments of Citharel, first that the labelled compound supplied by him was not in fact the product of photosynthetic activity in *Ascophyllum* and, second, that transport of metabolite could occur by secretion into the surrounding medium and reabsorption by *Polysiphonia*, or even by way of the mucilage surrounding the species. The outcome of Turner and Evans' experiments demonstrated that there was no translocation of the label in the tissues of *Ascophyllum* either with or without attached *Polysiphonia*. This means that if *Polysiphonia* is acting parasitically it can only exploit the cells in the immediate vicinity of the rhizoids, for the lack of translocation in the host will preclude any wider effect of such parasitism. They also demonstrated that *Polysiphonia* was able to accumulate  $^{14}\text{C}$  from labelled bicarbonate in the surrounding medium, confirming the independent carbon assimilation of the red alga, but they further showed that the 'epiphyte' was able to take up and use exogenously supplied labelled glucose.

So the question of whether or not *Polysiphonia lanosa* is a true epiphyte of *Ascophyllum nodosum* still remains unanswered. What is now clear is that if *Polysiphonia* depends upon its host

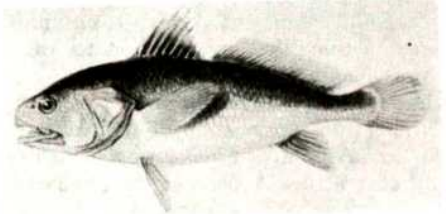
for more than support in a physical sense, it will be limited in its success by the lack of movement of metabolites in the host tissue and will be dependent on a local exploitation of cells or the absorption of materials secreted into the surrounding medium. As Turner and Evans point out, however, one cannot be sure that carbon is the sole object of any parasitism which occurs. Perhaps some other element is being sought, or even an organic growth factor. □

## Indo-Pacific drums reviewed

from a Correspondent

THE drums or croakers are fishes of very great commercial importance throughout tropical seas especially in shallow regions and where large rivers join the sea. They derive their common names from their habit of making loud noises of one kind and another; indeed it is claimed that native fishermen of South-east Asia only set their nets when their leader has heard the fish approaching the area. Because of their economic importance fishery workers in various countries have made numerous studies of their biology and aspects of their life history, and some have attempted taxonomic studies to establish some kind of phylogeny as well as a valid nomenclature. Notable amongst these was the work of Y. T. Chu, Y. L. Lo, and H. L. Wu, *A study of the classification of the sciaenoid fishes of China, with descriptions of new genera and species*, Shanghai Fisheries College, 1963, with a short English summary. Chu *et al.* produced a work of fundamental importance in which they placed considerable emphasis in classification on the structure of the otoliths and the swimbladder. Their synthesis proved to be a major landmark in understanding this group of fishes although necessarily it was largely confined to Chinese waters (where their commercial importance is very great).

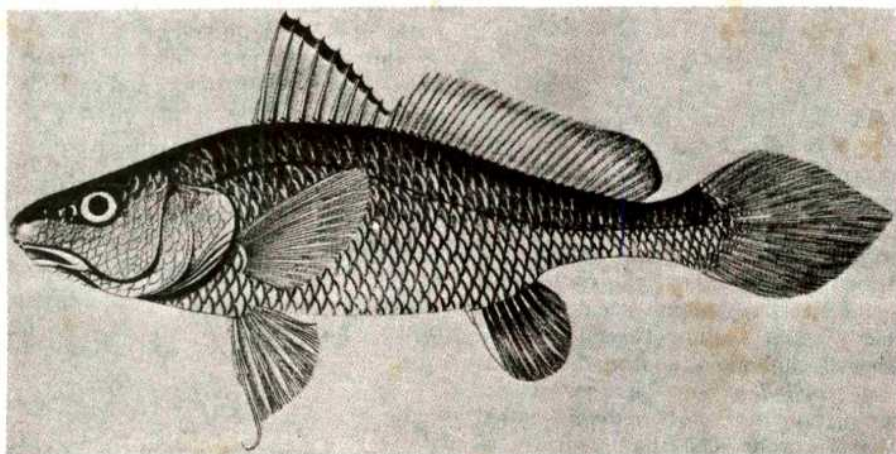
However, their work has now been amplified and refined by a major revision, *The sciaenid fishes (croakers or drums) of the Indo-West-Pacific* by



*Johnius (Johnieops) dussumieri* (Cuvier) (Cuvier *Le règne animal*, 1840).

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*Johnius coitor* (Hamilton *An account of the fishes of the Ganges*, 1822).

Ethelwynn Trewavas (*Trans. zool. Soc. Lond.* **33**, 253; 1977). Trewavas has expanded the area covered by major revisionary studies quite logically to include the less well known, but equally important, Indian Ocean species. Much of the new synthesis is founded on the form and structure of the swimbladder and otoliths for, having extended knowledge of these two organs, Trewavas finds them well found as major characters.

The degree of development of the swimbladder is a fascinating study and six main types are identified among the Indo-Pacific species. At its least elaborate the swimbladder is a simple large gas-filled sac with no appendages. Relatively few species (mostly Atlantic in their distribution) possess this form. Other types have diverticula running from the anterior end of the swimbladder and variously directed backwards or forwards (in one group running forwards into the head to underlie the skull), but in the subfamily Otolithinae the swimbladder is decorated with paired series of between 9 and 53 diverticula, mostly elegantly branched. In one tribe, Collichthyini, within this subfamily the diverticula are so developed that posteriorly their limbs enwrap the viscera, a situation which is paralleled in a distantly related West African family.

The large size of the otoliths of the sciaenid fishes is well known, both to science and to folklore (a necklace made of otoliths of a drum was found in a Californian Indian archaeological site), and names such as Otolithinae used within the group indicate that they have long been accepted as characteristic. Trewavas has extended the previous application of this feature to the taxonomy of the group, describing several new types and rationalising the nomenclature of the parts of the sagitta used by previous workers.

The net result of the analysis of otolith and swimbladder structure as

well as conventional taxonomic methods, such as fin ray, vertebra and scale counts, and morphological differences, is to produce a fundamentally important taxonomic work. In all, 65 species are recognised, grouped in 27 genera and 10 tribes. Each species is adequately described, with diagnostic

characters singled out, and basic synonymies are presented, with brief discussion on the synonymy. Keys are provided to all taxa as are diagnoses of families and genera. In total it presents fisheries workers in the Indian and West Pacific Oceans with a basic source for the identification of these important food fishes, one which was badly needed to judge from the synonymies presented.

It is peculiarly apt that these fishes which are well known for their sonic abilities have now been classified basically by the highly specific complex patterns of the swimbladder and the otolith and inner ear. It is generally accepted that the former is a sound-producing organ, of quality in this case, while the latter is concerned with sound reception. Living as they do in shallow tropical seas, especially in the clouded waters close to and within estuaries, the sciaenids have developed a sensitive auditory system which enables them to keep together and presumably to distinguish each other from other related species. □

## Animal migration at Tübingen

from Michael A. Bookman

A Symposium on Animal Migration, Navigation, and Homing was held in Tübingen, FRG from 12–20 August, 1977, in celebration of the 500th anniversary of the University of Tübingen.

ALTHOUGH the Sun can be used by many animals as a source of compass information, the ability to form a bright image on the retina and simultaneously derive accurate bearings without retinal damage remains puzzling. J. D. Pettigrew (California Institute of Technology) presented an interesting model linking the avian pecten oculi to solar orientation. Light scattered from the Sun's image on one edge of the retina would be interrupted by the pecten, which would cast a well-defined linear shadow over the temporal fovea adjacent to the image of the horizon. Peripheral imaging of the Sun prevents serious retinal damage while information is relayed to orientation-selective edge detectors in the visual wulst of the brain. With the potential for precise determination of the Sun's trajectory, some people were undoubtedly reminded of the 'rejected'

theories of bicoordinate solar navigation. An ingenious camera device that can be mounted on a pigeon's head to record the free-flying line of sight was described by K.-L. Köhler (Fürth, FRG). Investigation of orientation strategies with such a technique may aid the experimental evaluation of Pettigrew's model.

The sensory capabilities of homing pigeons were further reported on by M. L. Kriethen (Cornell University), J. D. Delius and J. Emmerton (Ruhr Universität, Bochum) and M. A. Bookman (Massachusetts Institute of Technology). In the laboratory, pigeons have demonstrated sensitivities to polarised light, ultraviolet light, small changes in barometric pressure, infrasound, and magnetic fields. Conditioning and electrophysiological experiments suggest that regional specialisation of the retina may optimise perception of polarised light. Sensitivity to ultraviolet light seems to result from the non-absorbance of the pigeon optical apparatus. L. J. M. Leask (Clarendon Laboratory, Oxford) proposed that the retina may also be involved in the detection of magnetic fields by a process of optical pumping and anisotropic decay of triplet states. Improved characterisation of magnetic responses, such as those by W. Wiltschko (Universität Frankfurt) with

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migratory birds, will help in the formulation and testing of possible sensory mechanisms. The influence of magnetic fields on orientation was further documented by C. Walcott (State University of New York, Stony Brook), who reported on the persistent disorientation of pigeons released near natural magnetic anomalies. The extent to which many of these sensory capabilities are made use of in navigation is unknown, although hypotheses abound.

The role of wind-carried odours in pigeon homing was discussed by F. Papi and S. Benvenuti (Università di Pisa), K. Schmidt-Koenig and J. Kiepenheuer (Universität Tübingen), and R. F. Hartwick (James Cook University, Queensland). Most investigators acknowledged that in some circumstances odours could provide directional cues; however, most data do not support a central role for odours in pigeon homing. In a later discussion, W. T. Keeton (Cornell) reasoned that just as a hierarchy of redundant cues seems to supply compass information, a similar hierarchy is probably used to obtain map information. Therefore, research should not aim to uncover 'the' navigational scheme, because several schemes are likely to be used in different conditions.

Odours, however, clearly occupy a central role in the spawning of salmon. A. D. Hasler and A. Scholz (University of Wisconsin, Madison) described a remarkable experiment in which hatchery-raised salmon were exposed to chemically scented water for 1 month before their release into Lake Michigan. During the subsequent spawning season, small streams were artificially scented with the same compounds. Imprinted salmon were then recovered in large numbers from the appropriately scented stream.

The sensitivity of marine sharks, skates, and rays to voltage gradients on the order of  $0.01 \mu\text{V cm}^{-1}$  was described by A. J. Kalmijn (Woods Hole Oceanographic Institute). In the laboratory, these fish oriented to magnetic fields, presumably by responding to local electric fields induced by swimming through the magnetic field. Theoretically, fish could monitor natural voltage gradients found within ocean currents to obtain compass information and determine the direction of water flow.

In experiments with hatchling sea turtles, M. Mrosovsky (University of Toronto) demonstrated that movement from the nest to the water's edge is governed by a phototropic response to an integrative right-left balancing of retinal and tectal stimulation. L. C. Ireland (Oakland University, Rochester) used ultrasonic transmitters to track hatchling turtles on the initial portion of their maiden ocean voyage.

As yearlings, the turtles reach distant feeding grounds. He concluded that the initial directional stimulus was simply 'away from land', which perhaps represented an extension of nest-to-water movement.

Whether migratory birds are capable of true bicoordinate navigation or predominantly utilise only simpler forms of compass orientation remains controversial. T. C. and J. M. Williams (Swarthmore College) reported that successful migrants moving between North and South America seem to maintain a Southeast heading during their entire autumn migration, and would therefore not require a bicoordinate system. S. A. Gauthreaux (Clemson University, South Carolina) observed inland daytime flights of nocturnal migrants which are felt to correct for night-time drift. These flights suggest that some nocturnal migrants may actually navigate during the early daylight hours. A new release and tracking procedure for migratory birds using balloons and radar was developed by S. T. Emlen and N. J. Demong (Cornell). Although complex, this technique combines experimental control with near-natural flight conditions. Initial experiments showed that sparrows were able to select meaningful headings when released under cloud layers that obscured celestial cues. □



## A hundred years ago

### Bees killed by Tritoma

In a friend's garden here where there are quantities of Tritomas or "red-hot-pokers," hundreds of bees have been this year destroyed by them. The honey produced by the flower is very abundant, and the bees enter the tube of the corolla to get at it; but the tube, which is only just large enough at the mouth, tapers gradually, and so the bee gets wedged in and cannot extricate itself. I saw numbers so caught, some in the fresh flower, while others remained in the completely withered and decaying blossoms. Perhaps it may be due to the fine warm days we have had this autumn, inducing the bees to work too late after our native honey-producing flowers have been destroyed by the wet and frosts; or is it a regular thing which happens every year? If so bee-keepers should discourage the Tritoma, or set to work to select varieties with flowers large enough not to kill their bees.

ALFRED R. WALLACE

From *Nature* 17, 15 November, 45; 1877.

## Plasma lipoprotein structure

from Angelo M. Scanu

A workshop on Lipoprotein Structure was held at the University of Chicago on 8-9 October, 1977.

DURING the past 10 years, many studies have been carried out on the structure of circulating lipoproteins, relying primarily on physical and chemical techniques. The workshop provided an assessment of the current state of knowledge on the subject.

The discussion on small-angle X-ray scattering was focused mainly on the low-density lipoproteins (LDL), where controversy still exists on the state of organisation of their surface components. The recent studies by V. Luzzati *et al.* (Gif-sur-Yvette, France) have led investigators to postulate the existence of 'bumps' or 'spikes', which were attributed to protein units protruding from the surface of the particles. The investigations of P. Laggner *et al.* (Graz, Austria), in turn, although yielding essentially similar X-ray results, were interpreted as being compatible with a surface having no convolutions. The organisation of the LDL core also came under sharp scrutiny. There was agreement on the presence, in this core, of cholesteryl esters and triglycerides in varying proportions; the concentric smectic lamellar organisation of the cholesteryl esters suggested by the studies of D. Atkinson and G. Shipley (Boston), was disputed, however, by Luzzati, who proposed an alternative organised micellar structure without, however, giving details. Agreement seems to exist on the notion that triglycerides influence the core organisation of cholesteryl esters; this view appears well documented by the results of studies on the LDL of animals with hyperlipidemias secondary to the administration of cholesterol-supplemented dietary fats.

Contrary to the rather extensive studies performed with small-angle X-ray scattering, the work on plasma lipoproteins carried out by neutron diffraction (H. B. Stuhmann, FRG) has been comparatively modest. The validity of the method was recognised, however, and further applications were anticipated with suitable replacement of some of the LDL constituents by their deuterated counterparts. An elegant study of the naturally occurring quasi-crystalline egg yolk lipoprotein system was presented by L. Banaszak (St. Louis University, St. Louis), who has reconstructed a two-fold symmetry model from electron micrographs of negatively-stained preparations.



Differential scanning calorimetry is proving its usefulness in the study of plasma lipoproteins. A systematic study of the thermal behaviour of LDL, HDL, and related systems was reported by D. Small (Boston), who obtained information on the phase behaviour of cholesteryl esters in the core of LDL particles. He also investigated the thermal stability of HDL, particularly that of its major protein, apolipoprotein A-I. R. Biltonen (University of Virginia, Charlottesville), using differential scanning calorimetry, estimated the statistical distribution of cluster sizes in phospholipid bilayers, and H. Pownall (University of Texas, Houston) applied microcalorimetric techniques to examine the association between apolipoproteins and lipid vesicles. Fluorescence spectroscopy was used by A. Jonas (University of Illinois, Urbana) in a study of lipoproteins and apoprotein-phospholipid-cholesterol recombinants; she proposed a bilayer structural model conceptually similar to that derived from X-ray and thermocalorimetric data. Pownall described the kinetics and mechanisms of lipid-amphiphile association, whereas Smith discussed an interesting application of an extrinsic fluorescence probe (pyrene), to the study of cholesterol exchange between lipoproteins using stopped flow kinetics.

The current status of  $^{13}\text{C}$ -NMR spectroscopy as applied to studies of the structure of plasma lipoproteins was summarised by E. Cordes (Indiana University, Bloomington). The results presented indicated that this technique is valuable in the study of the dynamics and phase transitions of surface and core lipids, particularly in combination with the analysis of model systems. J. Morrisett (Houston) reported results of his  $^{13}\text{C}$  and  $^{31}\text{P}$ -NMR spectroscopic studies on lipoprotein-X, an abnormal lipoprotein found in cholestasis. T. Glonek (University of Chicago) discussed the use of a chelated manganese paramagnetic ion to probe the accessibility and surface distribution of the phospholipids in both LDL and HDL.

One session was devoted to an examination of the mechanism of phospholipase  $\text{A}_2$  action and its use as a probe of the lipoprotein surface. F. Kézdy and colleagues (Chicago) found that the kinetic behaviour of this enzyme is similar for all of the major lipoprotein classes investigated; he therefore suggested that all of the phospholipase  $\text{A}_2$ -hydrolysable phospholipids represent a single pool at the surface of these particles. M. Wells (University of Arizona, Tucson) presented evidence that the physical state of the substrate influences the specificity of phospholipase  $\text{A}_2$  and pointed at the limited information available at present on the mechanism of action of

## Red hot sea

from Peter J. Smith

It is as certain as anything can ever be in the Earth sciences that the Red Sea is a region of very young seafloor spreading. Some of the evidence for this view comes from geothermal studies, which is only to be expected, although it comes as a surprise to be reminded by Girdler and Evans (*Geophys. J.* **51**, 245; 1977) that as recently as 1970 there were no more than 17 heat flow values available, 5 of which were only estimates from borehole temperature measurements. Since 1970, however, the quantity of data has increased fivefold, and Girdler and Evans have now been able to draw a much better, though still far from complete, picture of Red Sea geothermal characteristics.

The average heat flow for measurements made within 5 km of the Red Sea's deep water axis is  $467 \text{ mW m}^{-2}$ , or about eight times the world average of  $59 \text{ mW m}^{-2}$ , although individual values rise to more than 56 times the world average. Over the next 5-km interval the average then drops dramatically to  $89 \text{ mW m}^{-2}$ , presumably because of hydrothermal circulation; but it rises to  $140 \text{ mW m}^{-2}$  and  $165 \text{ mW m}^{-2}$  over succeeding 20 km intervals, settling to about  $111 \text{ mW m}^{-2}$  (still about twice the world mean) at distances of 50–170 km.

Thus heat flow is high not only in the axial zone but throughout the whole of the Red Sea. Indeed, both heat flow and temperatures ( $100^\circ\text{C}$  at

depths of less than 2 km) are so high along the edges of the sea that Girdler and Evans see geothermal heat as a potential source of power for coastal towns. But only 7 of the 86 individual heat flow values published by the end of 1975 lie below the world average; and as all come from the vicinity of the deep axial trough where heat flow is typically high, they would seem to reflect only transient thermal effects such as sediment slumping, or perhaps their positions above the downgoing limbs of hydrothermal circuits.

Sedimentary movement and hydrothermal circulation in the axial zone probably also explain the high variability of heat flow near the centre of the Red Sea. According to Girdler and Styles (*Nature*, **247**, 7; 1974) there have been at least two phases of seafloor spreading, the lithosphere produced by the second intruding and heating the older lithosphere and overlying evaporites; and although some people would dispute this precise interpretation, there seems every reason to suppose that evaporite movement has resulted from very recent spreading. Such complexity makes detailed interpretation of heat flow data difficult; but the general geothermal picture is evidently entirely consistent with the Red Sea as a young spreading zone. □

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the enzyme. L. Smith (Houston) discussed the mode of activation of a purified milk lipoprotein lipase preparation by fragments of apolipoprotein C-II, the specific activator of the enzyme, and identified regions considered to be specific for activation. He discussed a similar approach with lecithin-cholesterol acyl transferase, in which he used fragments of apolipoprotein C-I, one of the activators of the enzyme.

With regard to apolipoproteins, W. Fitch (University of Wisconsin, Madison) presented an elaborate study on structural predictions based on primary sequence data. The findings led to the recognition of striking internal homologies in apolipoprotein A-I, considered to originate from duplication and crossing over of an ancestral gene coding for an 11-residue repeat. A computer search for amphipathic helices in apolipoprotein was discussed by J. Segrest (University of Alabama, Birmingham), whose results support the hypothesis that these segments might

be responsible for lipid binding. G. Fasman (Brandeis University, Waltham) provided a lucid analysis of the role of  $\beta$ -turns in protein structure and of the experimental methods used for their detection. The importance of  $\beta$ -turns in apolipoprotein structure emerged from work by investigators at the University of Chicago, who presented the first full-scale space-filling model of human HDL $_3$ , based on available experimental data and on work in progress on the properties of the A apolipoproteins at the air-water interface. One major conclusion from these investigations was that all of the predicted structures ( $\alpha$ -helix, random coil, and  $\beta$ -turns) in apolipoprotein A-I and A-II are amphipathic, and that this unique property enables them to occupy predictable areas at the HDL surface. □

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# review article

## X-ray burst sources

Walter H. G. Lewin & Paul C. Joss\*

*More than 30 X-ray burst sources have been discovered in the past 2 years, and information on the observational properties of the burst emission is accumulating rapidly. The physical mechanism responsible for the bursts is so far undetermined, but the most promising models are based on the accretion of matter onto a compact object of Stellar mass.*

X-RAY bursts from a source in the globular cluster NGC6624 were discovered by Grindlay and Heise<sup>1</sup> in December 1975. Earlier, events had been reported that were believed to be hard X-ray bursts of cosmic origin<sup>2</sup>. However, after lengthy discussions between one of us (W.H.G.L.) and Dr Melioranski, we believe that these bursts and others reported by the same group<sup>3,4</sup> are not of cosmic origin but are probably caused by the Earth's magnetosphere.

In 1976 a further 19 X-ray burst sources were discovered, mostly by SAS-3 and OSO-8 (refs 5–7). To date (15 September 1977) the total number of burst sources is in excess of 30; they are strung along the galactic equator (Fig. 1) and the majority of them are located within  $\sim 35^\circ$  of the galactic centre<sup>7</sup>. At least two (but probably several more) are located in globular clusters<sup>8–12</sup>, and three burst sources have been tentatively identified with faint blue stars<sup>13–15</sup>. One of these identifications, proposed by McClintock, is almost certainly correct<sup>13,16</sup>.

A typical burst rises in less than a few seconds and lasts several seconds to minutes. In most bursts, the lower energy flux persists longer than the higher energy flux (Fig. 2). In at least three cases<sup>17–19</sup>, the time-dependent burst spectra can be fitted well by blackbody emission from a source with an effective radius of  $\sim 10$  km. If this interpretation is correct, there is good reason to believe that the burst sources are neutron stars or black holes of stellar mass.

The bursts are sometimes emitted at approximately regular intervals of from hours to days. At least eight burst sources emit a persistent (though variable) flux of X rays. No persistent emission has been detected from at least two burst sources<sup>5,20,21</sup>.

The mechanism which produces X-ray bursts is not yet known. There is little evidence to suggest that the burst sources are in binary systems, though many workers favour this idea. No proposed model has yet given a complete explanation for the behaviour of burst sources.

### Galactic distribution

If we adopt as an operational definition for X-ray bursts<sup>5</sup>: (1) rise time less than a few seconds, (2) duration from a few seconds to a few minutes, and (3) recurrence, then eighteen burst sources have been reported to date<sup>7,12</sup> (15 September 1977). Their positions are shown in Fig. 1. If we accept single burst-like events for which only the first two criteria were met, seven burst sources can be added (dashed lines and horizontal bars in Fig. 1). At least five more burst sources (not shown in Fig. 1) have been observed with SAS-3; their positions are very uncertain but they are probably all located within  $\sim 30^\circ$  of the galactic centre.

Many other burst-like events of cosmic origin that we omit have been reported in the literature; they are not known to meet either the rise time or the recurrence criterion. In this context we mention the intriguing results of the Los Alamos group<sup>22–24</sup>. It is very likely that some of the many events observed by them were X-ray bursts of the kind discussed here.

The burst sources are spread along the galactic equator and cluster near low galactic longitudes ( $|l| < 35^\circ$ ). The distribution is different from that of the known X-ray sources with 'steady' emission<sup>5,7</sup>. Eight burst sources (Table 1) have been identified with sources of steady emission (more will undoubtedly follow). This suggests that the burst sources form a subset of the steady X-ray emitters<sup>5,7</sup>. No steady emission has been observed from at least two sources<sup>5</sup>.

### Burst flux and recurrence

The observed peak intensities in bursts are typically  $\sim 10^{-8}$  to  $\sim 10^{-7}$  erg cm<sup>-2</sup> s<sup>-1</sup>. At source distances of  $\sim 10$  kpc, this would imply a peak luminosity of  $\sim 10^{38}$ – $10^{39}$  erg s<sup>-1</sup>.

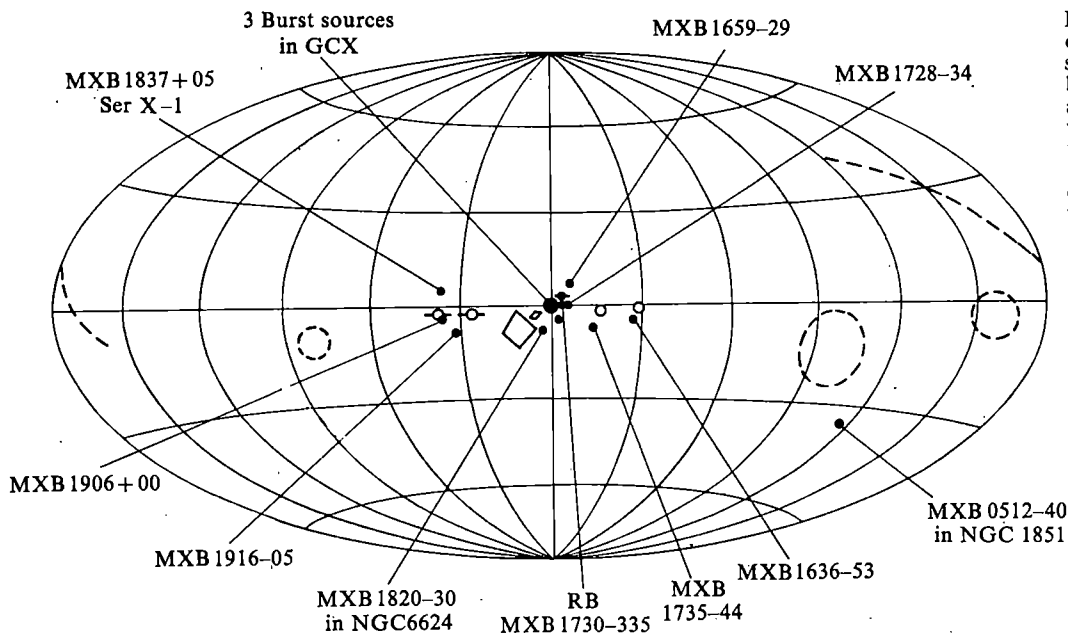
The total (integrated) flux of individual bursts differs by about 1–2 orders of magnitude from source to source. In general, the total flux of individual bursts from a given source does not change appreciably over a time scale of many burst intervals<sup>5,6</sup>.

Aside from the short rise times (less than a few seconds) and the longer decay times (several seconds to minutes), an important characteristic of X-ray bursts is their recurrence (not reported for  $\gamma$ -ray bursts). As first reported by Clark and his associates<sup>25</sup>, the recurrence is sometimes regular though not strictly periodic. The most regular burst intervals have been observed from MXB1659–29. During October 1976 the burst intervals from this source wandered slowly from 2.0 to 2.6 h, and the burst arrival times were often predictable to within several minutes<sup>5,20</sup>. Again, during the recent worldwide coordinated burst observations<sup>26</sup> in June and July 1977, SAS-3 detected a series of 17 bursts (intervals  $\sim 2.5$  h) with arrival times often (but not always) predictable to within 5 min. It may not be a coincidence that MXB 1659–29, which is the most 'regular' of all burst sources, does not emit a detectable flux of 'steady' X-rays<sup>5,20</sup>.

The apparent regularities in burst occurrence for several sources can persist for days<sup>5,6</sup>. During recent SAS-3 observations, however, we found that many sources that were believed to be regular actually become irregular on time scales of days to weeks. The most striking examples of highly irregular burst sources are MXB1837+05 (refs 27–29) and MXB1735–44 (ref. 30 and unpublished SAS-3 results); their burst intervals can vary from  $\sim 1$  h to  $\sim 2$  d.

The fact that most known burst sources have intervals from hours to days is probably the result of a selection effect due to the limitations of today's X-ray observatories; the longer the

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**Fig. 1** Sky map (galactic coordinates) of 18 X-ray burst sources which meet the three burst criteria (see text). Seven additional sources are shown for which the recurrence criterion of the bursts has not (yet?) been met. They are indicated with dashed lines (four sources) or with horizontal bars (three sources). Open error regions have areas  $\geq 3 \text{ deg}^2$ . The dashed line near the galactic anticentre represents an error box of  $\sim 120^\circ \times 2^\circ$ . The figure contains only burst sources reported before 15 September 1977. There are at least five additional known sources with poorly determined positions (see text). The names of some of the best studied burst sources are shown.

burst intervals, the smaller the chance of detection. It is likely that sources will be discovered with considerably longer burst intervals (weeks, months, years?). There are at present seven sources (Fig. 1) from which only one burst has been observed, yet some of these sources were observed continuously for many days. This may already be an indication that some of them have burst intervals in excess of several days. It is expected that in the era of the space shuttle, sensitive X-ray detectors will be flown with both large fields of view and the capability of determining source positions accurately. Such instruments would be ideal for the study of fast transient phenomena such as bursts.

It is thought that most burst sources have burst-active and inactive periods<sup>5,6</sup>. The most striking examples are MXB1820-30 (in NGC6624), MXB1730-335 (the rapid burster), MXB1743-28, and a burst source in Norma (refs. 5, 6, 21, 30-36). There is no indication that the periods of activity come at regular intervals, but this may well be the case for some sources. A possible (so far undetected) periodicity in the burst patterns or in the active and inactive periods could, in principle, uncover an orbital period of a binary system.

## Burst profiles

Bursts exhibit a large variety of shapes. There are bursts with spikes, peaks, bumps, shoulders and tails, and some show all these features<sup>5</sup>. Bursts from different sources usually look very different (Fig. 2). Bursts from one source, however, usually seem quite similar. Several burst sources have their own characteristic idiosyncracies, and we can often (but not always) tell by the burst profile from which source a burst came (Fig. 2). All eight bursts detected from MXB1743-29 (at  $\sim 35$ -h intervals) showed two (sometimes three) distinct peaks at energies above 6 keV (ref. 33). Bursts from MXB1735-44 are usually narrow, lasting only 4-6 s<sup>30</sup>. Bursts from MXB1636-53 and MXB1728-34 have fast initial decays (a few seconds) followed by long gradual decays at low energies<sup>18,19,37</sup>. There are cases, however where the bursts from one source look very different. Bursts from MXB1837+05 show great variation<sup>29</sup> which may be related to the irregularities in the burst intervals. Normally, bursts from MXB1728-34 occur quite regularly with intervals that wander slowly from  $\sim 4$  to  $\sim 8$  h<sup>18,37</sup>. But, in June 1977, during the worldwide coordinated burst observations<sup>28</sup>, only one burst was observed in 54 h, and the profile of this burst was very peculiar. During observations in July 1977 the bursts from MXB1728-34 again occurred regularly and seemed 'normal'. There is some observational evidence that

bursts which occur 'out of sequence' in a quasi-regular series have a different appearance from the other bursts in the series. This effect has been reported in bursts from MXB1636-53, MXB1916-05 and MXB1730-335 (the rapid burster) (refs. 19, 21, 38 and unpublished SAS-3 results).

Clark *et al.*<sup>31</sup> have observed 22 bursts from MXB1820-30 (in NGC6624). The burst intervals slowly wandered from  $\sim 3.4$  h to  $\sim 2.2$  h. The burst tails, which were clearly visible at the beginning of the observations, vanished gradually and were absent from the last few bursts. The burst activity came to a halt while the persistent emission from the source continued to increase.

## Burst spectra

Significant spectral changes occur during bursts. Typically the spectra soften considerably during burst decay (Fig. 2 and refs. 5, 6). The best examples are the bursts from MXB1743-29 (ref. 33), MXB1906+00 (ref. 39), MXB1735-44 (ref. 30 and unpublished SAS-3 results), MXB1728-34 (refs 18 and 37), MXB1636-53 (refs 19 and 40), MXB1916-05 (refs 41-43), MXB0512-40 (in NGC1851, ref. 10 and G. W. Clark, personal communication) and a source near  $l^{\text{II}} \sim 356^\circ.4$ ,  $b^{\text{II}} \sim 2^\circ.3$  (ref. 17). Spectral hardening during burst decay has only been observed in bursts from MXB1820-30 (refs 8, 25). Spectral hardening during the burst rise is quite common, however, and has been observed in bursts from MXB1728-34, MXB1636-53, MXB1820-30, MXB0512-40 and in a single burst from a source near  $l^{\text{II}} \sim 356^\circ.4$ ,  $b^{\text{II}} \sim 2^\circ.3$ . MXB1837+05 is special, as some bursts harden during the rise and others soften<sup>29</sup>.

Swank *et al.*<sup>17</sup> and Hoffman *et al.*<sup>18,19</sup> have found good blackbody fits to the spectra of bursts from three burst sources (MXB1728-34, MXB1636-53 and a source near  $l^{\text{II}} \sim 356^\circ.4$ ,  $b^{\text{II}} \sim 2^\circ.3$ ). During the early part of the bursts, the temperature increases to  $\sim 3 \times 10^7$  K. A cooling is then observed which continues for several minutes, after which there is no longer any detectable flux. If indeed the burst emission is blackbody radiation, the effective radius  $R$  of the emitting region can be calculated. Swank *et al.*<sup>17</sup> find that  $R \sim 100d$  km during the first 20 s of the burst and  $R \sim 15d$  km for the remainder of the burst, where  $d$  is the source distance in units of 10 kpc. Hoffman *et al.*<sup>18,19</sup> find for both MXB1728-34 and MXB1636-53 that  $R \sim 10d$  km. This size remains approximately constant throughout the bursts. These results suggest that, if the source distances are  $\sim 10$  kpc, then the bursts are produced by a neutron star or a black hole of stellar mass<sup>17-19</sup>.

Hoffman *et al.*<sup>19</sup> have discussed 'typical' bursts in terms of

two components: (1) an initial pulse which rises and falls sharply in a few seconds and is wider at higher energies, followed by (2) a long decay whose spectrum softens with time as the burst intensity gradually decreases (Fig. 2). They made the interesting observation that the initial pulse appears to be highly absorbed at low energies (1–3 keV) when a burst in a quasi-regular series arrives out of sequence (too early) (ref. 19 and unpublished SAS-3 results).

### Steady emission

It is quite common for a burst source to emit a persistent (but variable) flux of X-rays<sup>5,8</sup>. As shown in Table 1, high 'steady' fluxes ( $\sim 1/4$  that of the Crab Nebula) are observed from MXB1636–53 (refs 40, 44, 45), MXB1728–34 (refs 18 and 37), MXB1735–44 (ref. 30) and MXB1837+05 (refs 27–29). No steady flux has been observed from either MXB1730–335 (ref. 31) or MXB1659–29 (ref. 5).

Table 1 lists eight burst sources and the accurate SAS-3 positions of their associated steady sources (refs 46 and 47 and J. G. Jernigan, personal communication). It is highly probable that all associations in Table 1 are correct; the probability (based on the positional coincidences) that any one of them is wrong is  $\sim 1\%$ . It has been suggested<sup>33</sup> that the three burst sources near the galactic centre produce a steady emission that may account for part of the emission of GCX (ref. 54). There are a few more burst sources for which a candidate steady source has been proposed<sup>12, 34, 48, 53</sup>.

The ratio of time-averaged luminosity of the steady emission to that of the burst emission varies from  $\lesssim 2$  (rapid burster) to  $\sim 250$  (ref. 19). If we exclude the rapid burster, the lowest value is  $< 25$  for MXB1659–29 (unpublished SAS-3 results). These ratios are important in testing nuclear-flash models of X-ray bursts (see section on theoretical models).

### Optical identifications

There are optical candidates<sup>13–18</sup> for three burst sources (MXB1837+05, MXB1636–53 and MXB1735–44). All three are faint ( $\sim 18$  mag) blue stars. At least one star, recently suggested by McClintock, is almost certainly a correct identification<sup>13, 18</sup>.

Very accurate positions for the steady sources that produce bursts have recently been reported by the Ariel 5 group in Birmingham<sup>61</sup> and the SAS-3 group<sup>46, 47</sup>. It is expected that as a result more optical identifications will soon follow.

The burst sources MXB1820–30 and MXB0512–40 are located in the globular clusters NGC6624 (refs 8, 25, 31) and NGC1851 (refs 10 and 11), respectively. The rapid burster<sup>21</sup> (MXB1730–335) is almost certainly located in 'Liller's' globular cluster (refs 5, 6, 9, 55), and a burst source with a positional accuracy of  $\sim 1^\circ$  may be located in the globular cluster NGC6441 (ref. 12). Thus, a very high fraction of the seven known X-ray emitting globular clusters<sup>56</sup> also emit bursts, and it is expected that more burst sources will be found in globular clusters. The large majority of burst sources, however, (Fig. 1) are not located in visible globular clusters<sup>5–7</sup>.

### Spectra and character of associated steady sources

The spectra of the persistent X-ray emission of burst sources do not fit a blackbody model and are almost always softer than the mean burst-spectra<sup>5</sup> (integrated over the first  $\sim 10$  s of the burst). The only exception is 4U1915–05 (MXB1916–05), for which a hard spectrum ( $kT > 35$  keV) has been reported for the steady emission<sup>43</sup>.

At Ostriker's suggestion<sup>57</sup>, Cominsky (personal communication) has compared 'colour-colour' plots of the steady X-ray emission from eight burst sources (the hard source 4U1915–05 was not included) with that of other types of X-ray source (five globular clusters, seven strong galactic centre sources, one pulsating low-mass binary, two non-pulsating low-mass binaries, seven high-mass binaries, and two black hole candidates). She concludes that the colour-colour plots are similar for: (1) the burst sources (steady emission), (2) the globular cluster X-ray sources, (3) the strong galactic centre sources, and (4) the non-pulsating low-mass binaries (Sco X-1 and Cyg X-2). The colour-colour plots, however, are different for the high-mass binaries, the pulsating low-mass binary (Her X-1) and the two black hole candidates (Cyg X-1 and Cir X-1). This suggests that the first four categories may have very similar mechanisms for producing X-rays and that they may well all be binaries. But, if the burst sources are binaries, one may wonder why none of them shows any evidence of their binary character. On the other hand, it is very difficult to deduce a possible binary membership from X-ray data if no pulsations or eclipses are observed (as in Sco X-1 and Cyg X-2) and if the approximate binary period is not already known (for example, from optical observations). It may well be that the possible binary character of X-ray burst sources will first be found in optical data. It is expected that the optical

Table 1 Steady X-ray sources from which bursts have been observed\*

Burst source	Steady source	Positions of steady sources from SAS-3 (refs. 46, 47 and J. G. Jernigan, personal communication)						Error circle radius†	Intensity (Uhuru counts s <sup>-1</sup> ; ref. 48)		Refs.
		RA (1950)	Dec. (1950)	h	min	s	"		Maximum	Minimum/maximum	
MXB0512–40 (in NGC1851)	2S0512–400 2A0512–399 4U0513–40	05	12	29	–40	05	53	20"	18	3	10, 11, 48–50
MXB1636–53 (blue star)	2S1636–536 4U1636–53	16	36	57.6	–53	39	21	20"	250	2	14, 19, 40, 45, 47, 48, 51
MXB1728–34	2S1728–337 4U1728–33	17	28	39.6	–33	47	52	30"	150	5	5, 6, 18, 37, 47, 48, 51, 52
MXB1735–44 (blue star!)	2S1735–444 4U1735–44	17	35	19.5	–44	25	22	20"	210	1.7	13, 16, 30, 47, 48, 51
MXB1820–30 (in NGC6624)	2S1820–303 4U1820–30	18	20	28.4	–30	23	14	20"	320	3	1, 5, 6, 8, 25, 31, 32, 48, 51
MXB1837+05 (blue star?)	2S1837+049 4U1837+04	18	37	29.8	04	59	23	20"	280	2	5, 6, 27–29, 46, 48
MXB1906+00	Ser X-1 2S1905+000 A1905+00	19	05	54.9	00	05	37	35"	4.05 $\pm$ 1.1‡	–	5, 6, 39, 46, 48
MXB1916–05	4U1857+01 2S1916–053 4U1915–05	19	57	02.9	11	34	14	20"	20	2	41–43, 46, 48

\*These eight associations are quite reliable (see text). Four others<sup>12, 34, 48, 53</sup> (not in this table) have been proposed but are uncertain.

†Each error circle is an approximate 90% confidence region.

‡Average intensity.



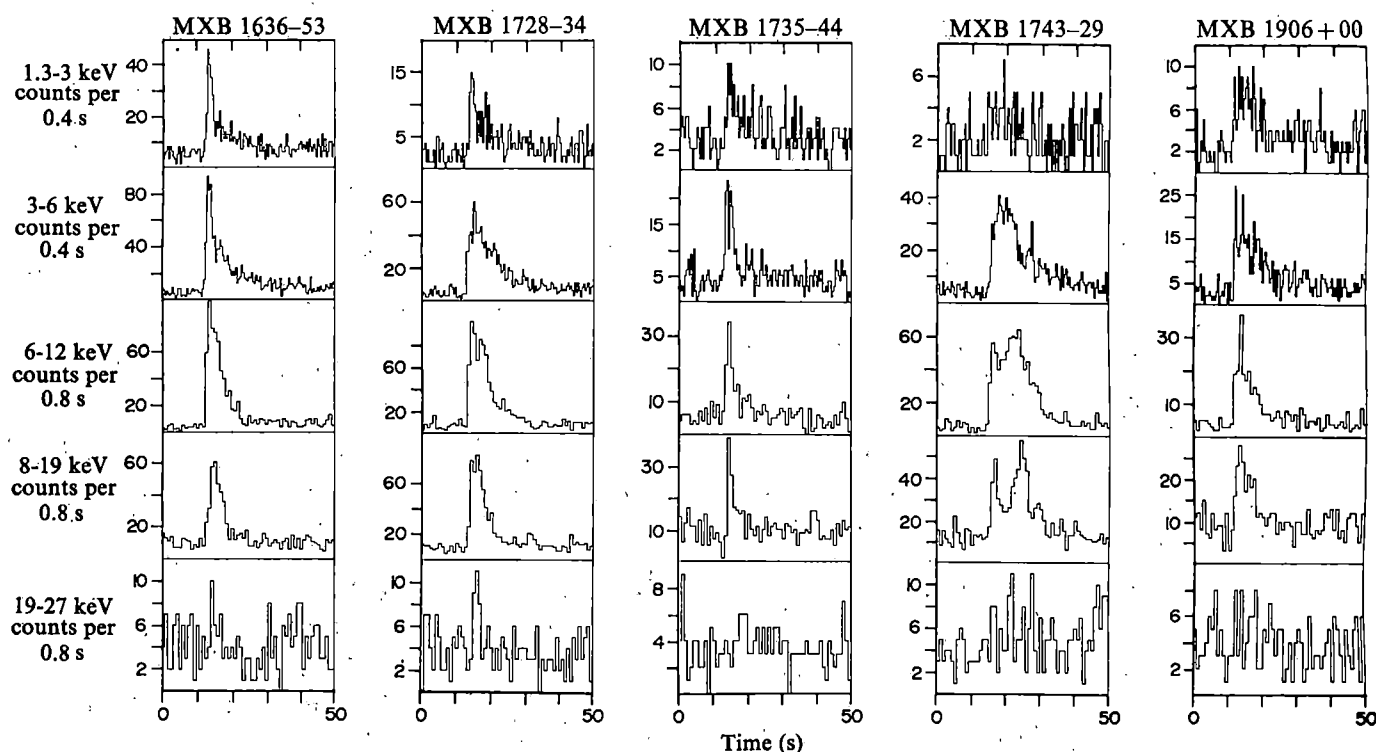


Fig. 2 Profiles (five energy channels) of bursts from five different sources (SAS-3 data). Note that in all cases the gradual decay (tail) persists longer at lower energies than at higher energies. The burst profiles are quite distinctive for each particular source (see text).

candidates<sup>13-16</sup> will be carefully examined for evidence of binary characteristics.

### The rapid burster

The rapid burster (MXB1730-335) is quite unlike all other burst sources<sup>21</sup>. It can produce up to 4,000 bursts per day<sup>6</sup>, the integrated energy  $E$  of individual bursts can vary by two orders of magnitude, and  $E$  is approximately proportional to the time  $\Delta t$  for the next burst to occur<sup>21</sup>. A more detailed analysis has shown that the linear relation breaks down at low burst energies, where the waiting time to the following burst is considerably longer than one would expect from a strictly linear relation<sup>6</sup>.

Occasionally, 'anomalous' bursts have been observed that occur more or less independently of the rapidly repetitive ('normal') burst sequence, grossly violating the  $E-\Delta t$  relation<sup>38</sup>. These 'anomalous' bursts in general rise more slowly, and their maximum luminosity is  $\sim 1/3$  that of the maximum observed luminosity in 'normal' bursts<sup>38</sup>.

The rapid burster is almost certainly located in a globular cluster<sup>9,55</sup>, whose position is inside the small error circle (1.8 arc min radius) for the burst source<sup>6,51,58</sup>. As noted above, no steady emission has been observed from this source; the ratio of time averaged steady flux to time averaged burst flux is  $\lesssim 2$  (ref. 21), which is uniquely low<sup>5,6</sup>. This source was burst-active in March-April 1976, in April-May 1977 and in September 1977 (refs 21, 30, 35, 36, 59). Bursts from this source have also been detected with Uhuru<sup>48</sup>.

The time averaged burst luminosity varied significantly (by factors of  $\sim 2-3$ ) during March and April 1977<sup>5</sup>. The latest SAS-3 results, obtained by H. Marshall (personal communication) show a hint of a period of about 0.8 d in the time averaged burst luminosity.

### Comparison with $\gamma$ -ray bursts

The most striking differences between X-ray bursts and  $\gamma$ -ray bursts<sup>60</sup> are: (1) X-ray burst sources cluster along the galactic equator whereas  $\gamma$ -ray burst sources do not; (2) unlike  $\gamma$ -ray bursts, X-ray bursts are repetitive on time scales of hours to

days; (3) the spectra of  $\gamma$ -ray bursts are much harder than those of X-ray bursts; and (4) the total flux density of a  $\gamma$ -ray burst is typically  $\sim 2$  orders of magnitude higher than that of an X-ray burst. The  $\gamma$ -ray bursts cannot be the high-energy tails of normal X-ray bursts produced by nearby burst sources; the absence of recurrence excludes this possibility. However, some generic relation between  $\gamma$ -ray bursts and X-ray bursts cannot be excluded<sup>34</sup>. It is interesting to note that a  $\gamma$ -ray burst (from galactic latitude  $\sim -46^\circ$ ) observed from Apollo 16 shows a triple-peaked time structure<sup>61</sup>, which is remarkably similar to the structure in bursts from MXB1743-29 (Fig. 2 and ref. 33).

### Theoretical models

Most speculations concerning the nature of X-ray burst sources fall into two broad categories: (1) instabilities in the accretion of matter onto a compact object (white dwarf, neutron star, or black hole), and (2) thermonuclear flashes in matter accreted onto the surface of a neutron star. Theoretical models based on these ideas are still rather primitive.

Among possible instabilities in accretion onto a compact object of roughly solar mass, most models are based on interchange instabilities at the Alfvén surface of a neutron star or white dwarf. Within this category, Lamb *et al.*<sup>62</sup> have investigated the possibility of a thermal instability in spherical accretion onto a magnetised neutron star, while Svestka<sup>63</sup>, Henriksen<sup>64</sup>, Baan<sup>65</sup> and Joss and Rappaport<sup>66</sup> have discussed the possible role of orbital motion of the accreting matter in producing an instability. Wheeler<sup>67</sup> and Liang<sup>79</sup> have investigated instabilities within an accretion disk surrounding a compact object. These preliminary discussions are a promising start. The physics of the proposed instability mechanisms is very complex, however, and no completely satisfactory model has yet been established.

Grindlay and Gursky<sup>68</sup> and Grindlay<sup>69</sup> have presented the idea that the burst sources may be associated with massive ( $\gtrsim 10^3 M_\odot$ ) black holes. A specific mechanism for producing the burst emission was suggested by Bahcall and Ostriker<sup>70</sup>, who invoked a compact object in orbit about a massive black hole,

which interacts with an accretion disk that surrounds the hole. The latter idea is by now excluded; we now know that the burst intervals are often very irregular, unlike the highly periodic behaviour that one would expect from this model.

The introduction of massive black holes was largely motivated by the association of 3U1820-30, the earliest discovered burst source, with the globular cluster NGC6624 (ref. 8). This cluster is one of five globular clusters which have highly condensed cores and which contain sources of persistent X-ray emission<sup>66</sup>. It had previously been suggested<sup>71,72</sup> that these steady X-ray sources may be massive black holes undergoing accretion within the cluster cores. However, as more burst sources were discovered, it was found that the distribution of these sources is very different from the apparently spherical distribution of globular clusters about the galactic centre (Fig. 1 and refs 5-7). This fact, together with the absence of visible globular clusters in the direction of many burst sources, now makes it almost certain that the majority of burst sources are not located in globular clusters.

Another motivation for the massive black hole model was the spectral hardening observed during the decay in bursts from NGC6624 (refs 8, 25), which was ascribed to Compton scattering of the emitted X rays by a very hot ( $\sim 10^9$  K) gas cloud that is gravitationally bound by a massive black hole<sup>68</sup>. Canizares<sup>73</sup> has shown, however, that the observed hardening could be produced by scattering in a cooler cloud, which might be bound by a compact object of mass  $\lesssim 5M_\odot$ . Moreover, bursts from most other sources, including the one located in the globular cluster NGC1851, soften during their decay (Fig. 2, refs 5, 6 and G. W. Clark, personal communication). As pointed out earlier<sup>5,6</sup>, it seems that little evidence is left to support the conjecture that X-ray bursts are associated with massive black holes.

Maraschi and Cavaliere<sup>74</sup> and Woosley and Taam<sup>75</sup> have suggested thermonuclear flashes in the surface layers of accreting neutron stars as a totally different alternative mechanism for the production of X-ray bursts. The gravitational energy released per unit mass of matter falling onto a neutron star of mass  $M$  and radius  $R$  will be  $(GM/R) \sim 10^{-1} c^2$ , while that released by very rapid (timescale  $< 10$  s) thermonuclear fusion of any nuclear fuel will be  $\lesssim 10^{-3} c^2$  (refs 76, 77). Hence, this type of model predicts that the ratio,  $\alpha$ , of time averaged persistent accretion-driven X-ray luminosity to time averaged burst luminosity should exceed  $\sim 10^3$ . This criterion is apparently met by some burst sources with rather bright steady X-ray counterparts (Table 1 and refs 5, 6). However, it apparently fails in the case of the rapid burster, for which  $\alpha \lesssim 2$  during times of burst activity<sup>21</sup>. It also seems to fail for MXB1659-29, whose observational properties are similar to those of most other burst sources (unlike the rapid burster) but which has recently been found to have  $\alpha < 25$  during burst-active times (unpublished SAS-3 results). Thus, it seems that this mechanism can account for no more than a subset of the presently known burst sources. In any event, a complete evaluation of the physics of thermonuclear flashes in neutron-star surface layers has yet to be carried out.

If the burst sources, are in fact, compact objects of moderate mass, then they may be accreting matter from close binary stellar companions. In this respect, they may be very similar to other binary X-ray sources, which are also widely believed to consist of compact objects that are undergoing accretion from relatively normal companion stars (see ref. 78 and references therein). If the burst sources are in such systems, one might expect to find periodic temporal variability in the associated steady X-ray emission or in the optical candidates. As noted above, however, no such periodic variability has been reported to date.

The blackbody spectra reported for three burst sources (see section on burst spectra and refs 17-19) are the only direct indication to date that burst sources are neutron stars or black holes of stellar mass.

The mechanism which produces X-ray bursts is thus not yet known, but the most promising mechanisms are based on the

accretion of matter onto a compact object of Stellar mass.

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**Note added in proof:** Recently, Hoffman, Marshall and Lewin<sup>80</sup> discovered that the rapid burster occasionally produces 'special' bursts which, unlike the rapidly repetitive bursts, show the typical characteristics of bursts from other sources (similar time scale of burst intervals and similar spectral evolution during burst decay). They suggest in a recent preprint that rapidly repetitive bursts may be a replacement of the persistent emission observed from many other burst sources and that in this special case the persistent emission manifests itself as bursts. If this were so, it opens the interesting possibility that the rapidly repetitive bursts may be the result of instabilities in the accretion flow (gravitational potential energy) but that the 'special' bursts from the rapid burster and the X-ray bursts from all other sources are the result of thermonuclear flashes.

1. Grindlay, J. & Heise, J. *IAU Circ.* No. 2879 (1975).
2. Babushkina, O. P. et al. *Sov. Astron. Lett.* 1, 32 (1975).
3. Sagdeev, R. Z. *IAU Circ.* No. 2959 (1976).
4. Babushkina, O. P. et al. *Sov. Astr. Lett.* 1, 115 (1975).
5. Lewin, W. H. G. *Proc. Eighth Texas Symposium on Relativistic Astrophysics*, *Ann. N.Y. Acad. Sci.* (in the press).
6. Lewin, W. H. G. *Mon. Not. R. astr. Soc.* 179, 43 (1977).
7. Lewin, W. H. G. et al. *Nature* 267, 28 (1977).
8. Grindlay, J. et al. *Astrophys. J. Lett.* 205, L127 (1976).
9. Liller, W. *Astrophys. J. Lett.* 213, L21 (1977).
10. Clark, G. W. & Li, F. K., *IAU Circ.* No. 3092 (1977).
11. Forman, W. & Jones, C. *Astrophys. J. Lett.* 207, L177 (1976).
12. Li, F. & Clark, G. W. *IAU Circ.* No. 3095 (1977).
13. McClintock, J. E. *IAU Circ.* No. 3084 (1977).
14. McClintock, J. E. *IAU Circ.* No. 3088 (1977).
15. Davidsen, A. *IAU Circ.* No. 2824 (1975).
16. Bond, H. E. *IAU Circ.* No. 3085 (1977).
17. Swank, J. H. et al. *Astrophys. J. Lett.* 212, L73 (1977).
18. Hoffman, J. A., Lewin, W. H. G. & Doty, J., *Mon. Not. R. astr. Soc.* 179, 57P (1977).
19. Hoffman, J. A., Lewin, W. H. G., Doty, J., Jernigan, J. G. & Haney, M. *Astrophys. J. Lett.* (in the press).
20. Lewin, W. H. G., Hoffman, J. A. & Doty, J. *IAU Circ.* No. 2994 (1976).
21. Lewin, W. H. G. et al. *Astrophys. J. Lett.* 207, L95 (1976).
22. Belian, R. D., Conner, J. P. & Evans, W. D. *Astrophys. J. Lett.* 207, L33 (1976).
23. Belian, R. D., Conner, J. P. & Evans, W. D. *IAU Circ.* No. 2969 (1976).
24. Evans, W. D., Belian, R. D. & Conner, J. P. *Astrophys. J. Lett.* 207, L91 (1976).
25. Clark, G. W. et al. *Astrophys. J. Lett.* 207, L105 (1976).
26. *IAU Circ.* No. 3078 (1977).
27. Swank, J. H., Becker, R. H., Pravdo, S. H. & Serlemitsos, P. J. *IAU Circ.* No. 2963 (1976).
28. Li, F. K. & Lewin, W. H. G. *IAU Circ.* No. 2983 (1976).
29. Li, F. K. et al. *Mon. Not. R. Astr. Soc.* 179, 21P (1977).
30. Lewin, W. H. G., Hoffman, J. A., Doty, J., Li, F. K. & McClintock, J. E. *IAU Circ.* No. 3075 (1977).
31. Clark, G. W. et al. *Mon. Not. R. Astr. Soc.* 179, 651 (1977).
32. Lewin, W. H. G., Doty, J., Hoffman, J. A. & Li, F. K. *IAU Circ.* No. 2984 (1976).
33. Lewin, W. H. G. et al. *Mon. Not. R. astr. Soc.* 177, 83P (1976).
34. Grindlay, J. & Gursky, H. *Astrophys. J. Lett.* 209, L61 (1976).
35. White, N. E. & Burnell, S. J. *IAU Circ.* No. 3067 (1977).
36. Lewin, W. H. G. & Hoffman, J. A. *IAU Circ.* No. 3079 (1977).
37. Hoffman, J. A. et al. *Astrophys. J. Lett.* 210, L13 (1976).
38. Ulmer, M. P., Lewin, W. H. G., Hoffman, J. A., Doty, J. & Marshall, H. *Astrophys. J. Lett.* 214, L11 (1977).
39. Lewin, W. H. G. et al. *Mon. Not. R. astr. Soc.* 177, 93P (1977).
40. Swank, J. H., Becker, R. H., Pravdo, S. H., Saba, J. R. & Serlemitsos, P. J. *IAU Circ.* No. 3000 (1976).
41. Swank, J. H., Becker, R. H., Pravdo, S. H., Saba, J. R. & Serlemitsos, P. J. *IAU Circ.* No. 3010 (1976).
42. Lewin, W. H. G., Hoffman, J. A. & Doty, J. *IAU Circ.* No. 3087 (1977).
43. Becker, R. H. et al. *Astrophys. J. Lett.* 216, L101 (1977).
44. Giacconi, R. et al. *Astrophys. J. Suppl.* 27, 37 (1974).
45. Hoffman, J. A., Doty, J. & Lewin, W. H. G. *IAU Circ.* No. 3025 (1977).
46. Doxsey, R. E., Apparaio, K. M. V., Bradt, H. V., Dower, R. G. & Jernigan, J. G. *Nature* 269, 112 (1977).
47. Jernigan, J. G., Apparaio, K. M. V., Bradt, H. V., Doxsey, R. E. & McClintock, J. E. *Nature* (in the press).
48. Forman, W. et al. *Astrophys. J. Suppl.* (in the press).
49. Markert, T. P. & Clark, G. W. *IAU Circ.* No. 2735 (1974).
50. Cooke, B. A. et al. *Mon. Not. R. Astr. Soc.* (in the press).
51. Wilson, A. M., Carpenter, G. F., Eyles, C. J., Skinner, G. K. & Wilmore, A. P. *Astrophys. J. Lett.* 215, L111 (1977).
52. Lewin, W. H. G. *IAU Circ.* No. 2922 (1976).
53. Tananbaum, H., Chaisson, L. J., Forman, W., Jones, C. & Matilsky, T. A. *Astrophys. J. Lett.* 209, L125 (1976).
54. Kellogg, E., Gursky, H., Murray, S., Tananbaum, H. & Giacconi, R. *Astrophys. J. Lett.* 169, 199 (1971).
55. Kleinmann, D. E., Kleinmann, S. G. & Wright, E. L. *Astrophys. J. Lett.* 210, L83 (1976).
56. Clark, G. W. *Highlights of Astronomy*, 4, (in the press).
57. Ostriker, J. P., *Proc. Eighth Texas Symposium on Relativistic Astrophysics*, *Ann. N.Y. Acad. Sci.* (in the press).
58. Carpenter, G. F., Skinner, G. K., Wilson, S. M. & Wilmore, A. P. *Nature*, 262, 473 (1976).
59. Joss, P. C., Ricker, G., Mayer, W., & Hoffman, J. *IAU Circ.* No. 3108 (1977).
60. Klebesadel, R. W. & Strong, I. B. *Astrophys. Space Sci.* 42, 3 (1976).
61. Metzger, A. E., Parker, R. H., Gilman, D., Peterson, L. E. & Trombka, J. I. *Astrophys. J. Lett.* 194, L19 (1974).
62. Lamb, F. K., Fabian, A. C., Pringle, J. E. & Lamb, D. Q. *Astrophys. J.* 217, 197 (1977).
63. Svestka, J. *Astrophys. Space Sci.* 45, 21 (1976).
64. Henriksen, R. N. *Astrophys. J. Lett.* 210, L19 (1976).
65. Baan, W., *Astrophys. J.* 214, 245 (1977).
66. Joss, P. C. & Rappaport, S. *Nature*, 265, 222 (1977).
67. Wheeler, J. C. *Astrophys. J.* 214, 560 (1977).

68. Grindlay, J. & Gursky, H. *Astrophys. J. Lett.* 205, L131 (1976).  
 69. Grindlay, J. E. *Astrophys. J.* (in the press).  
 70. Bahcall, J. N. & Ostriker, J. P. *Nature*, 262, 37 (1976).  
 71. Bahcall, J. N. & Ostriker, J. P. *Nature*, 256, 23 (1975).  
 72. Silk, J. & Arons, J. *Astrophys. J. Lett.* 200, L131 (1975).  
 73. Canizares, C. R. *Astrophys. J. Lett.* 207, L101 (1976).

74. Maraschi, L. & Cavaliere, A. *Highlights of Astronomy* 4, (in the press).  
 75. Woosley, S. E. & Taam, R. E. *Nature*, 263, 101 (1976).  
 76. Joss, P. C. *Nature* (in the press).  
 77. Lamb, D. Q. & Lamb, F. K. *Astrophys. J.* (in the press).  
 78. Rappaport, S. & Joss, P. C. *Nature* 266, 123 (1977).  
 79. Liang, E. P. T. *Astrophys. J.* 218, (in the press).  
 80. Hoffman, J. A., Marshall, H. & Lewin, W. G. H. *IAU Circ. No.* 3117 (1977).

## articles

# Oxygen isotope and palaeomagnetic evidence for early Northern Hemisphere glaciation

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*Oxygen isotope and palaeomagnetic analysis of the lower half of LDGO piston core V28-179 shows that glacial-interglacial fluctuations have characterised Earth's climate for the past 3.2 Myr, before which there was a period of stable 'interglacial' or 'preglacial' climate. The scale of glaciations increased about 2.5 Myr ago.*

THE stratigraphically longest detailed oxygen isotopic records from the oceans extend to sediments about 2.1 Myr old<sup>1,2</sup> and indicate Pleistocene-like glacial events well below the horizon stratigraphically equivalent to the base of the Quaternary as it is defined in Italy<sup>3</sup>. Here we extend the isotopic record and identify the onset of these quasi-cyclic glacial-interglacial fluctuations. For this we required a core of sediment that accumulated somewhat more slowly than in the area of our previous studies.

Cores from the Equatorial Pacific were described by Hays *et al.*<sup>4</sup>, who showed that north of the belt of maximum biological productivity along the equator it is possible to take piston cores containing a record extending well into the Pliocene. They discussed the lithostratigraphy and magnetostratigraphy of several cores, and the biostratigraphic record for foraminifera, diatoms and radiolaria (expanded in refs 6 and 7) while Gartner<sup>5</sup> presented the nannofossil stratigraphy. Since then several cruises have recovered cores from the same general area. Of these we selected V28-179 (collected by N.D.O.), for isotope analysis because it contained the thickest accumulation of sediment for the Gauss magnetic epoch.

Core V28-179 was taken at 4° 37' N, 139° 36' W in 4,509 m water depth. The core comprises alternating layers of foraminiferal chalk ooze, marl ooze and marl with some dominantly diatomaceous layers. Most of the core is intensely burrowed, and many of the plugs of sediment washed for analysis could be seen to contain components of slightly different colour mixed

Fig. 1. Magnetic record for core V28-179, 150 Oe and interpretation in terms of the standard palaeomagnetic polarity scale.

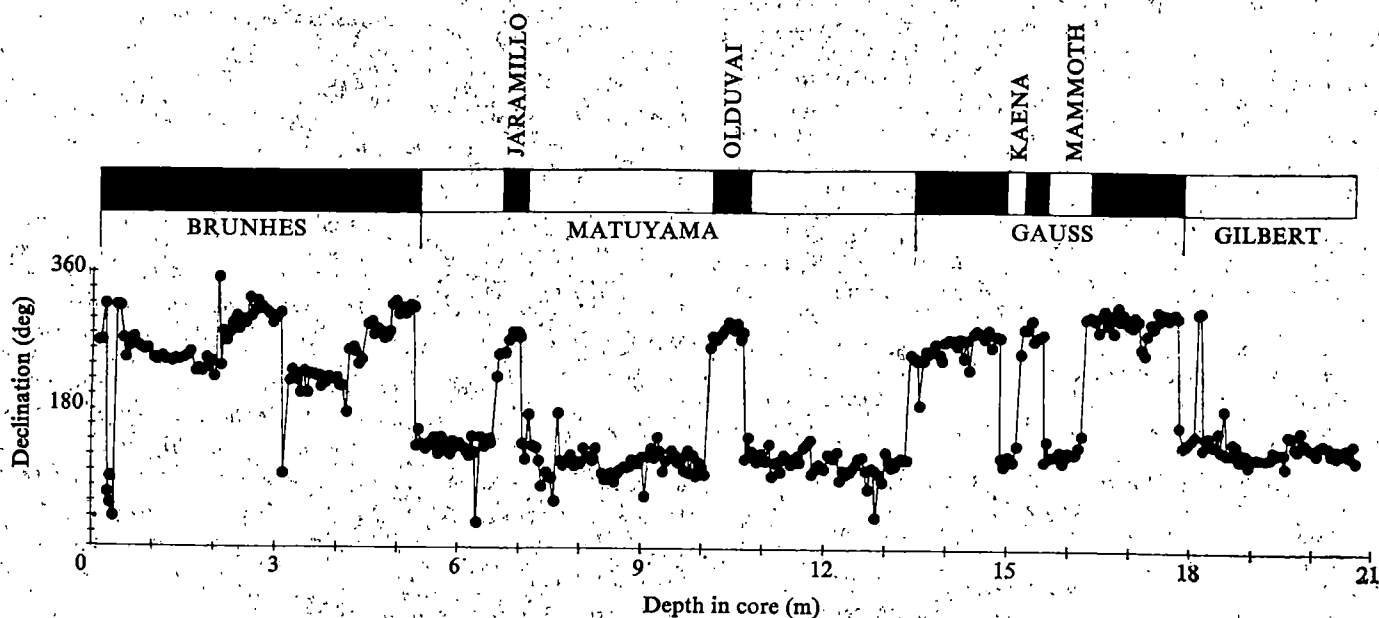


Table 1 Magnetic boundaries V28-179

Brunhes-Matuyama	527±2.5 cm
Matuyama-Jaramillo	657±4 cm
Jaramillo-Matuyama	700±2.5 cm
Matuyama-Olduvai	1,007±2.5 cm
Olduvai-Matuyama	1,068±3 cm
Matuyama-Gauss	1,358±2.5 cm
Gauss-Kaena	1,488±2 cm
Kaena-Gauss	1,517±2.5 cm
Gauss-Mammoth	1,558±2 cm
Mammoth-Gauss	1,622±2.5 cm
Gauss-Gilbert	1,779±2.5 cm
Unnamed short interval (1,808-1,817±2.5 cm)	

together by burrowing. This strongly suggested that the depth through which burrowing took place was frequently greater than the typical thickness of a single layer deposited under uniform environmental conditions. We assume, following evidence already available<sup>9</sup>, that the chief source of variability in the sediment derives from climatically controlled variations in the proportion of carbonate remaining in the sediment. As these variations occurred on a depth scale that was smaller than the depth of plainly imperfect homogenisation by burrowing organisms, it shows that we are limited to an investigation of the general character of the ocean oxygen isotopic record within the good time-framework provided by the palaeomagnetic record of the core.

The record of changing ocean oxygen isotopic composition in the Late Pleistocene has been investigated in some detail in cores with accumulation rates up to about 8 cm kyr<sup>-1</sup> (ref. 10), and it has been shown that some cores with accumulation rates of over 3 cm kyr<sup>-1</sup> preserve a sufficiently good record to allow the identification of the effect of the changing tilt of the Earth's orbit (period 40 kyr) and of precession of the equinoxes (period about 23 kyr)<sup>11</sup>. To investigate the Lower Pleistocene in a piston core, it is necessary to work in an area of rather lower sediment accumulation. We have already discussed<sup>1</sup> the loss of information which characteristically occurs under these conditions. Even if the mixing in the sediment of core V28-179 were homogeneous, climatic fluctuations with a 40 kyr period would have a wavelength in the sediment of only about 20 cm and would be scarcely detectable. Since the mixing is clearly not homogeneous, and since several samples proved too low in carbonate for an analysis to be made at all, it is likely that the detailed character of the climatic record is inaccessible. What we can do is to identify the date of inception of the isotopic fluctuations which have characterised at least the whole of the Pleistocene<sup>1</sup>. Although there are other types of evidence which indicate periods of climatic deterioration, there is as yet no information pertaining to the date of the earliest accumulation in the Northern Hemisphere of continental ice sheets that would have been of sufficient scale to produce a detectable effect on the oxygen isotopic composition of the oceans<sup>12</sup>.

The core was sampled for magnetic measurement at 5 cm intervals along its length. The measurements were performed on a slow speed fluxgate magnetometer of the type described by Molyneux<sup>13</sup>. In order to ascertain the proper alternating field to use for blanket demagnetisation and to determine the magnetic stability of the core 10 samples spaced throughout the core were progressively demagnetised in peak alternating fields of up to 250 Oe. The magnetic stability was found to vary throughout the length of the core with values of the median destructive field ranging from 25 to 225 Oe. Natural remanent magnetic intensities ranged from 0.2 to 20 × 10<sup>-6</sup> e.m.u. cm<sup>-3</sup>. A field of 150 Oe peak value was selected for blanket demagnetisation. The resulting plot of declination change against depth is shown in Fig. 1 plotted relative to an arbitrary fiducial mark scribed on the ship during the extrusion process<sup>14</sup>. The correlation of the resulting magnetic pattern to the standard time scale is easily done since the reversals are clearly delineated. This correlation has been confirmed by foraminiferal studies<sup>15</sup>

using the foraminiferal zonation previously determined for equatorial Pacific sediments<sup>4,6,7</sup>.

An important fact is that the pattern of coiling changes in *Pulleniatina* and in *Globorotalia tumida* shows that the lowest reversely magnetised section of sediment below about 18m can only represent the interval from the Gilbert-Gauss boundary to a horizon somewhat later than the Cochiti event; the bottom of the core probably has an age between 3.5 and 3.6 Myr (ref. 15).

Table 2 Oxygen and carbon isotope data for *Globocassidulina subglobosa* from core V28-179

depth δ(cm)	<sup>18</sup> O	<sup>13</sup> C
1,011	+4.30	-1.44
1,031	+3.79	-1.35
1,051	+4.03	-1.24
1,061	+3.52	-1.10
1,070	+3.67	-0.73
1,080	+4.21	-1.05
1,090	+3.89	-0.87
1,129	+3.86	-0.85
1,140	+4.14	-1.14
1,150	+3.72	-0.83
1,170	+3.48	-0.67
1,180	+4.31	-1.11
1,190	+3.77	-0.62
1,220	+3.79	-1.24
1,241	+3.80	-0.85
1,271	+4.15	-1.29
1,280	+3.46	-0.96
1,291	+4.02	-1.28
1,310	+4.30	-1.61
1,321	+3.65	-0.98
1,341	+3.98	-1.40
1,351	+3.99	-0.87
1,361	+3.41	-0.54
1,370	+4.05	-1.10
1,381	+3.94	-1.08
1,391	+3.29	-0.65
1,420	+3.92	-0.69
1,442	+3.55	-0.85
1,450	+3.77	-0.92
1,480	+4.02	-1.04
1,510	+3.42	-0.67
1,520	+3.87	-0.92
1,530	+3.36	-0.60
1,580	+3.93	-0.96
1,590	+3.80	-1.18
1,600	+3.92	-1.01
1,610	+3.35	-0.92
1,620	+3.76	-0.61
1,631	+3.69	-0.88
1,660	+3.66	-0.94
1,670	+3.60	-0.74
1,680	+3.52	-0.68
1,690	+3.13	-0.78
1,700	+3.50	-0.88
1,711	+3.16	-1.04
1,721	+3.19	-0.57
1,731	+3.19	-0.71
1,741	+3.37	-0.53
1,771	+3.13	-0.70
1,780	+3.43	-0.42
1,791	+3.53	-0.38
1,800	+3.14	-0.33
1,810	+3.56	-0.88
1,820	+3.19	-0.47
1,830	+3.28	-0.55
1,840	+3.30	-0.73
1,870	+3.25	-0.48
1,890	+3.20	-0.15
1,900	+3.41	-0.91
1,911	+3.29	-0.82
1,920	+3.20	-0.51
1,950	+3.40	-0.39
1,940	+3.24	-0.83
1,961	+3.36	-0.96
1,990	+3.40	-0.79
2,030	+3.30	-0.67
2,040	+3.51	-0.62
2,051	+3.45	-0.76
2,060	+3.14	-0.96



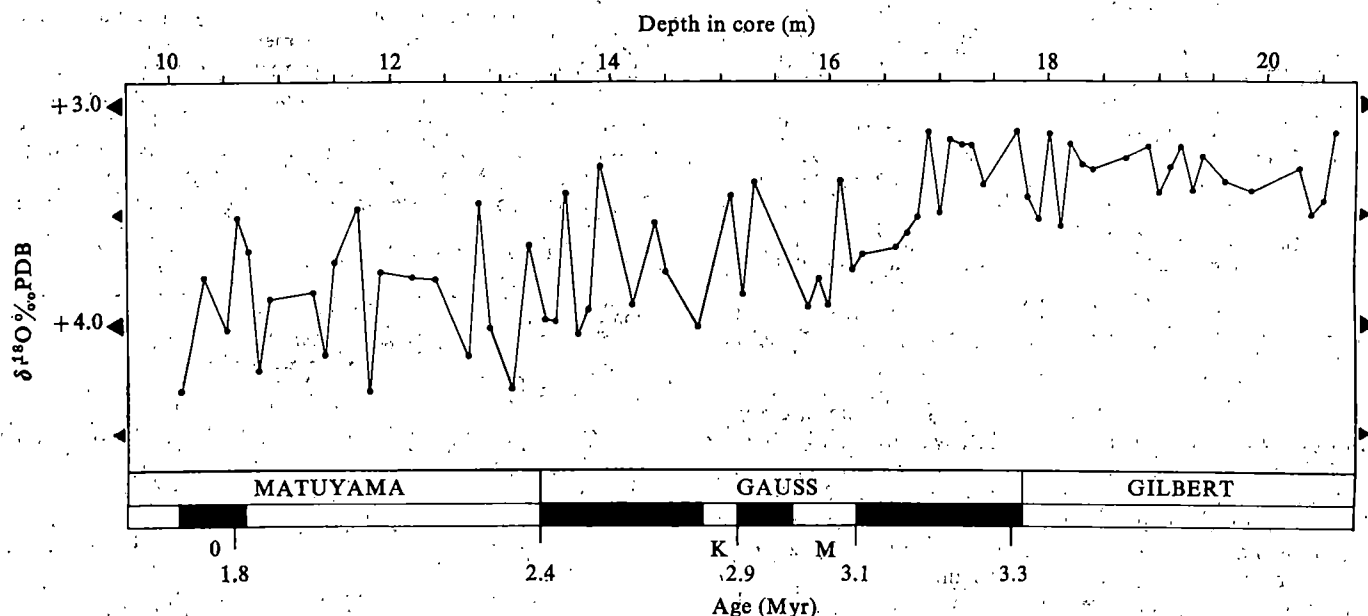


Fig. 2 Oxygen isotopic composition of *Globocassidulina subglobosa* in core V28-179 from 10m to 21m. *G. subglobosa* living on the sea floor at the coring site today would have an  $^{18}\text{O}$  content of about +3.50‰. Magnetic events are indicated by O (Olduvai), K (Kaena) and M (Mammoth). Ages indicated are based on current estimates of the chronology for the magnetic record<sup>26</sup>.

The placement within the core of the major palaeomagnetic transitions are given in Table 1. The only departure of the magnetic stratigraphy from the standard magnetic time scale is the presence of a short normally magnetised interval at 1,800 cm, below the Gauss normal magnetic epoch. It is possible that this represents a short reversal of the magnetic field since the core does not show any apparent physical disturbance at this level.

Oxygen isotope analyses were made at 10 cm intervals in the samples previously used for magnetic studies. The samples were dispersed in distilled water in a mechanical shaker, and after brief ultrasonic treatment, they were sieved on a 125  $\mu\text{m}$  mesh and dried in a cool oven on the sieves. Foraminifera for isotopic analysis were taken from this fraction further cleaned ultrasonically and purified by roasting *in vacuo* at 400 °C for 30 min. Carbon dioxide was released from the carbonate by the action of 100% orthophosphoric acid at 50 °C and analysed in a VGMicromass 602C mass spectrometer; analyses are reported to the PDB standard on the basis of multiple analyses of circulating standard carbonates.

The preservation of foraminifera is very poor at the water depth at which core V28-179 was taken (4,509 m). It was, therefore, generally not possible to obtain for analysis *Globigerinoides sacculifer*, the species used in our previous studies<sup>1,8</sup>. Instead, we chose to analyse benthonic foraminifera, which are less susceptible to dissolution. As many species of benthonic foraminifera do not deposit their carbonate in isotopic equilibrium with the sea water they inhabit<sup>18</sup>, it would clearly be desirable to analyse a single species throughout. *Uvigerina*, known to deposit carbonate in isotopic equilibrium, is extremely rare, but in the majority of samples we were able to extract sufficient specimens of *Globocassidulina subglobosa* for analysis.

To calibrate *G. subglobosa* for possible departure from isotopic equilibrium, we analysed the top part of core V19-28 from which we obtained excellent isotopic data for *Uvigerina*<sup>10,17</sup>. Our measurements show that *G. subglobosa* may be even closer to isotopic equilibrium than *U. senticosus*, the species analysed in core V19-28. Comparisons of the oxygen isotopic composition of *G. subglobosa* and of *U. spinulosa* in Oligocene samples confirms the suitability of this species for oxygen isotope work<sup>18,19</sup>. The bottom temperature at the site of core V19-28 is about 1.8 °C; at the site of V28-179 it is about 1.1 °C. The mean  $^{18}\text{O}$  content of *Globocassidulina subglobosa* in the upper part of core V19-28 is +3.30‰ (five analyses); using the relationship  $T = 16.9 - 4.4\delta + 0.10\delta^2$  (refs 20, 21) enables us to estimate that the temperature difference between

the two sites should be equivalent to an isotopic difference of about 0.2 ‰, so that the expected value at the V28-179 site is about +3.50 ‰. Since the sediment accumulation rate at the coring site is only about 0.6 cm kyr<sup>-1</sup>, the core top would be likely to contain a mixture of glacial and recent specimens and would not be useful for calibration purposes.

Analytical results for *G. subglobosa* are given in Table 2, and are plotted in Fig. 2. The record may best be described as a caricature of the true course of events, in that it is certainly not possible to derive an accurate record of events in such gradually accumulating sediment. It is, however, quite clear that the uppermost 17 m of the core are predominantly glacial in character; below 17 m, isotopic values are consistently close to the present-day value. Taking this lower section first, we may conclude that between about 3.5 Myr and 3.2 Myr ago no big ice sheets accumulated in the Northern Hemisphere. Isotopically, the ocean was in an interglacial state, with more or less constant isotopic composition. The standard deviation among the measurements for this part of the core is 0.13 ‰. If this were entirely due to real variability, it would represent glacial events of a magnitude up to the equivalent of a 26 m range in eustatic sea-level fluctuation<sup>8</sup>. The variations are on so small a scale that if they derive from real glacial events, then they could be caused by changes in the Antarctic ice sheet, or by small Northern Hemisphere glaciations. It is conceivable, however, that this variability stems entirely from analytical error. More accurate measurements in a section with more rapid sediment accumulation will be needed to investigate possible small-scale glacial events earlier than 3.2 Myr ago.

Just below the Mammoth event at 1,680 m we see a positive excursion in isotopic composition that is well above possible analytical uncertainty and is of such a magnitude (0.4 ‰) as to represent about a 40 m sea-level equivalent in stored ice (assuming its isotopic composition was about -35 ‰). This date is in substantial agreement with the inception of ice-rafting observed in sediments from site 116 in Leg 12 of the Deep-Sea Drilling Project<sup>22</sup> and with evidence of glaciation in Iceland<sup>23</sup> and with ref. 12, but the present data give for the first time the scale of fluctuations which have continued ever since.

At the base of the Matuyama (sample at 1,310 cm) a glacial isotopic excursion of about 1.0 ‰ is observed. This is a characteristic extreme value over the lower Matuyama section. In deep-Pacific cores with a high accumulation rate the maximum difference between glacial and interglacial isotopic values in

the late Pleistocene is about 1.60 per mil (ref. 10). Thus we have clear evidence that glaciations of a magnitude of at least two-thirds that of the late Pleistocene glacial maxima were occurring in the time interval from 2.5 to 1.8 Myr ago. This was probably the scale of glaciation throughout the Lower Pleistocene<sup>1</sup>. Evidently a major change in the character of glaciations occurred at about 2.5 Myr ago. The substantial carbon isotopic event at that point in the record may represent a large drop in the continental biomass<sup>24</sup> and may have been associated with significant floral extinctions under severe environmental pressure<sup>25</sup>.

Few localities are likely to preserve a sea-level record for the past 3 Myr. But, it may be deduced from Fig. 2 that the last lengthy period of stable sea level and low ice volume terminated about 3.2 Myr ago, so that it is likely that a major constructional coral terrace feature is present in some areas dating from this time; marine highstands in the remaining Pliocene, like those of the Pleistocene, were probably brief. On the basis of this analysis one might advance the hypothesis that before 3.2 Myr ago, continental environments were relatively stable over a long period of time; since then the Earth has been subjected to the stress of continually fluctuating climate, with pleasant climates like our own occurring infrequently and lasting only about 10,000 years.

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1. Shackleton, N. J. & Opdyke, N. D. *Geol. Soc. Am. Mem.* 145, 449-464 (1976).
2. van Donk, J. *Geol. Soc. Am. Mem.* 145, 147-164 (1976).
3. Haq, B. U., Berggren, W. A. & van Couvering, J. A. *Nature* (in the press).
4. Hays, J. D., Saito, T., Opdyke, N. D. & Burckle, L. H. *Geol. Soc. Am. Bull.* 80, 1481-1514 (1969).
5. Gartner, S. *Geol. Soc. Am. Bull.* 84, 2021-2034 (1973).
6. Saito, T., Burckle, L. H. & Hays, J. D. in *Late Neogene Epoch Boundaries* (eds Saito, T. & Burckle, L. H.) 226-244 (Am. Mus. nat. Hist., New York, 1975).
7. Saito, T. *Geology* 307-309 (1976).
8. Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* 3, 39-55 (1973).
9. Arrhenius, G. *Swed. Deep-Sea Exped. Rep.* 5, 6-227 (1952).
10. Shackleton, N. J. *Phil. Trans. R. Soc. B280*, 169-179 (1977).
11. Hays, J. D., Imbrie, J. & Shackleton, N. J. *Science* 194, 1121-1132 (1976).
12. Shackleton, N. J. & Kennett, J. P. in *Init. Rep. DSDP* 29, 801-807 (1975).
13. Molyneux, L. *Geophys. J. R. Astr. Soc.* 24, 1-5 (1971).
14. Opdyke, N. D. *Rev. Geophys. Space Phys.* 10, 213-249 (1972).
15. Sciarrillo, J. R., thesis, Rutgers Univ. (1976).
16. Duplessy, J. C., Lalou, C. & Vinot, A. C. *Science* 168, 250-251 (1970).
17. Ninkovich, D. & Shackleton, N. J. *Earth Planet. Sci. Lett.* 27, 20-34 (1975).
18. Boersma, A. & Shackleton, N. J. *Init. Rep. DSDP* 39 (in the press).
19. Boersma, A. & Shackleton, N. J. *Init. Rep. DSDP* 40 (in the press).
20. O'Neill, J. R., Clayton, R. N. & Mayeda, T. K. *J. Chem. Phys.* 51, 5547-5558 (1969).
21. Shackleton, N. J. *Colloques Int. Cent. natn. Rech. scient.* 219, 203-210 (1974).
22. Berggren, W. A. *Init. Rep. DSDP* 12, 953-963 (1972).
23. McDougall, I. & Wensink, H. *Earth Planet. Sci. Lett.* 1, 232-236 (1966).
24. Shackleton, N. J. in *The Fate of Fossil Fuel CO<sub>2</sub> in the Oceans* (eds N. R. Andersen & A. Malahoff) Plenum, London and New York, 1977).
25. Zagwijn, W. H. *Boreas* 3, 75-97 (1974).
26. Klitgord, J. L., Heustis, S. F., Mudie, J. D. & Parker, R. L. *Geophys. J. R. Astr. Soc.* 43, 384-424 (1975).

## Immunological specificity and memory in a scleractinian coral

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*Tissue transplantation immunity with a specific memory component is demonstrated in populations of Montipora. This highly discriminating immunoreactivity derives from extensive allogeneic polymorphism of histocompatibility (H) markers. An H system of immunorecognition is postulated to have originated in multicellular invertebrates probably beginning with coelenterates.*

POSSESSION of a specific immune system, including immunorecognition leading to selectively inducible responses with a memory component, has long been considered unique to vertebrates. Although invertebrate defence reactions were thought to lack sharp specificity, we now know that metazoans ranging from coelenterates to protochordates possess a well-developed capacity for recognition of foreign tissue followed by antagonistic reactions. Among higher invertebrates, immunorecognition at the allogeneic level has been repeatedly described in annelid worms<sup>1,2</sup>, echinoderms<sup>3,4</sup>, and tunicates<sup>5,6</sup>. Convincing evidence of allogeneic incompatibility in coelenterates has been reported from controlled experiments with colonial hydroids<sup>7</sup>, gorgonians<sup>8</sup>, and anthozoans, including sea anemones<sup>9,10</sup> and corals<sup>11,12</sup>. The situation in sponges, the most primitive of multicellular animals, is less clear. Certain species exhibit allogeneic recognition yielding non-fusion of separate clones after an initial period of adhesion, but cytotoxic reactions have not been reported<sup>13</sup>.

Continuing scepticism concerning the existence of immunocompetence in invertebrates<sup>14,15</sup> focuses on ques-

tions of specificity, memory and the molecular basis of cytotoxic reactivity. A specific alloimmune memory component to transplantation immunity among higher invertebrates has already been documented in annelids<sup>16</sup> and in echinoderms<sup>4</sup>. In living corals, specific reactivity is demonstrated by consistent compatibility of intracolony or syngeneic transplants, while intercolony allografts and interspecific xenografts are invariably incompatible. These distinct reactions, characteristic of many scleractinian corals, are both naturally-occurring on tropical reefs and reproducible in laboratory experiments<sup>11,17</sup>. Quasi-immune surveillance in corals, with manifestations ranging from mild to severe, is evidenced by contact avoidance reactions, allogeneic contact incompatibility, chronic xenogeneic incompatibility and acute interspecific cytotoxicity. Positive recognition of foreignness in the form of non-fusion in areas of tissue contact precedes cytotoxic reactions which usually require a longer period of sensitisation. Examination of allogeneic graft reactions in *Montipora verrucosa*, a foliaceous reef-building coral abundant in the Hawaiian Islands, surprisingly suggested a specific immunorecognition system with a memory component of at least short-term duration<sup>12</sup>. We now present an extensively controlled study of the essential immunocompetence of this coelenterate as a function of polymorphism of histocompatibility (H) markers, specificity and memory.

### Experimental tissue grafts

*M. verrucosa* is most attractive for tissue grafting because of its flat configuration and rapid healing. Many techniques for placing pieces of coral in firm, soft-tissue contact have



Fig. 1 Intracolony isografts of *M. verrucosa* showing compatible fusion at interface (arrows) at 30 d after grafting. Technique of pair grafting on plexiglas base using monofilament nylon tie-downs is also illustrated. Approximately  $\times 1$ .

been tried. Notched plexiglas rectangles about  $23 \times 12 \times 1$  cm and high-test tie-downs of monofilament nylon proved adequate for grafting paired *Montipora* pieces ranging in dimensions from 9–72 cm<sup>2</sup> (Fig. 1). Heavy bone shears were used to cut pieces to desired size, but only uncut edges were joined as graft interfaces. Grafts were established by tying together laterally two pieces of living coral with 50 pound test monofilament nylon on plexiglas plates, using small lead crimps to bind the nylon firmly. All coral was maintained in a rapidly running and unfiltered seawater system at 24–26 °C; in these conditions at the University of Hawaii Facility at Leiliwi Bay, *Montipora* thrived for for many months. Coral pieces were kept immersed in seawater in large trays while grafting and scoring.

Tissue reactions at transplant interfaces were scored under a large platform stereomicroscope, usually at  $45\times$  magnification. Useful indicators of graft viability versus rejection were (1) allogeneic contact avoidance/inhibition evident by failure of soft tissues to fuse or grow across small gaps; (2) persistence or disappearance of pigmented zooxanthellae in coenenchyme at contact points; (3) hyperplasia/blanching/death of soft tissue in contact zones. Cytotoxic reactions of allogeneic incompatibility may be either unilateral or bilateral when first observed, but nearly all became bilateral in *Montipora*. For purposes of quantitating cytotoxic reaction times of allografts, zones of soft tissue death of 1 mm or more from either side of the interface were scored as definitive. This extent of tissue death is unequivocal when measured under the stereomicroscope (Fig. 2) and allowed different investigators to score the same grafts on successive days with objectivity. Initial allocytotoxic reactions of 0.5 mm or less may take 2–3 weeks to become definitive in occasional slow reactors.

Technical trauma sometimes causes slight tissue destruction ( $<0.5$  mm) that is evident within 24 h of establishing grafts, but which heals within 1 week after grafting. Unless otherwise stated, all isografts, first-set and repeat-set allo-

grafts in the present experiments were 12–16 cm<sup>2</sup> in area. Soft tissue to a depth of 1–2 mm overlies the hard calcareous skeleton. Other technical considerations have been described elsewhere<sup>12,17</sup>. Three widely separate populations of *M. verrucosa* from the island of Hawaii were used. Specific first- and second-set allografts involved comparison of Kapoho and Onekahakaha populations, and unrelated third-party allografts were from the City of Refuge reef. Time–percentage effect curves, reaction time ratios and slope function ratios were determined by nomographical methods<sup>18</sup>.

### Evidence of alloimmune specificity and memory

Control intracolony isografts were always compatible as shown by soft tissue fusion in all contact areas as early as 3–5 d after grafting. Neither blanching nor tissue hyperplasia accompanied this fusion in some 18 pairs tested. By 12–15 d, coenenchyme continuity with confluent zooxanthellae was observed throughout contact zones. Calcification was already well underway at interfaces, but gaps of 3 mm or more remained open. Smaller gaps disappeared as a result of bilateral outgrowth. By 24–30 d, complete reconstitution indistinguishable from normal ungrafted coral was evident (Fig. 1). This compatible fusion then persisted indefinitely.

Rejection of initial or first-set allografts was preceded by a lag or sensitisation period of 2–3 weeks during which time intimate soft tissue contact was established. Antagonistic reactions were then revealed by progressive blanching and loss of soft tissue in the immediate contact area. In a series of 32 test pairs, primary allografts yielded a median reaction time (MRT) of 22.0 (19.2–25.3) d. Secondary allografts, established by resetting the same coral pairs at new interfaces distant from the original contact area, showed accelerated and intensified cytotoxic reactions with an MRT of 11.6 (9.4–14.4) d. This MRT difference with an interval of 40 d between first-set and second-set grafting (Table 1)

Fig. 2 Intercolony allografts of *Montipora verrucosa* showing bilateral cytotoxic incompatibility restricted to immediate contact zone. Note blanching and soft tissue death at interface (arrows). Approximately  $\times 1$ .

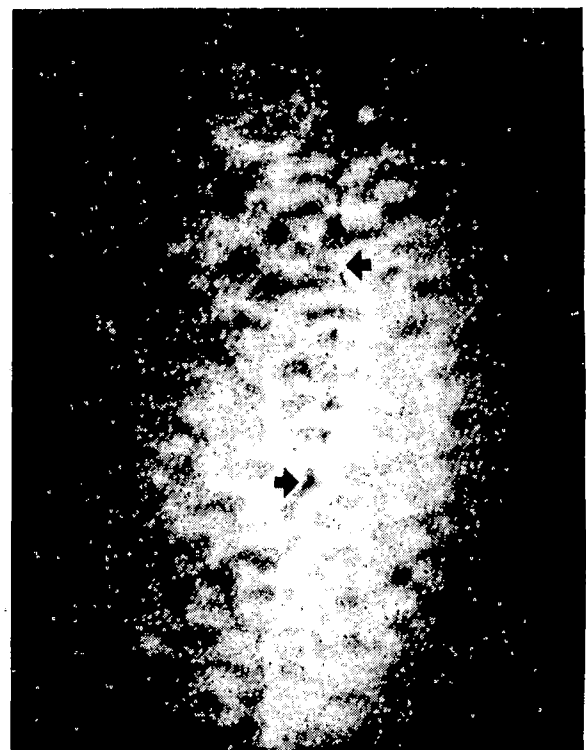


Table 1 Evidence of specificity and memory in allograft reaction times of *Montipora verrucosa*

Allograft expt	No. coral pairs scored	Median reaction times (MRT) (d)*	Range of individual cytotoxic reaction times (d)	Interval between 1° and 2° grafting (d)§	Comment
First-set	32	22.0 (19.2–25.3) (9.2)	16–40	—	
Second-set	18	11.6 (9.4–14.4) (5.7)	9–23	40	MRT difference between 1° and 2° grafts highly significant†
Unrelated third-party Biphasic‡	21				
(1) Slow reactions	8	22.9 (18.4–28.5) (7.4)	23–35	41	MRT not significantly different from 1°; specific induced immunity not evident
(2) Rapid reactions	13	13.2 (9.7–18.0) (8.2)	8–17	41	MRT not significantly different from 2°; induced immunity is evident

\*Ranges in parentheses are 95% confidence limits; single numbers in parentheses below each entry are the standard deviations of the MRT's.

†Slopes of curves for first- and second-set reactions were parallel within experimental error, suggesting that same qualitative events occurred, but at different rates.

‡MRT difference between slow and fast reactors is significant at 95% confidence level. Note also that these two groups were qualitatively distinctive: slow reactions were concurrently bilateral, whereas rapid reactions were unidirectional as expected with one-way pre-immunisation.

§1°, First-set and 2°, second-set grafting.

was significantly suggestive of immune memory. Intensified second-set reactivity was evidenced by accentuated early hyperplasia and secretion of mucus at interfaces.

To test for specificity, 21 pairs of third-party grafts from a geographically distant City of Refuge population were performed with first-set rejectors from either Kapoho or Onekahakaha sources. These third-party grafts yielded a broad range of cytotoxic reaction times reflecting a biphasic distribution of accelerated and non-accelerated responses (Table 1). The rapid reactions (61%) were conspicuously unilateral, restricted to the third-party grafts as expected for unidirectional pre-immunisation. Conversely, the remaining slow reactions, not significantly different from first-set reactions, were concurrently bilateral and typical of primary reactions (Fig. 3). In other words, third-party grafts were distinguishable qualitatively on the basis of unilateral versus bilateral reactivity as well as quantitatively on the basis of cytotoxic reaction times. These striking results reveal both specificity in transplantation alloimmunity and extensive antigenic polymorphism among genetically separate *M. verrucosa*. The impressive polymorphism of H markers in this species is also confirmed by the incompatibility of all of some 280 intercolony graft combinations tested thus far within and between Onekahakaha, Kapoho and City of Refuge populations.

To what extent does allograft size influence direction, rate of appearance or degree of severity of cytotoxic reactions? Allografting in pairs at tissue mass ratios of 2:1, 4:1, and 8:1 was accomplished with the smaller piece the same size (9 cm<sup>2</sup>) in three experiments (Table 2). Within this range, graft dosage had little or no effect on the direction or timing of allograft reactivity. Initial cytotoxic reactions were scored with equal frequency on small or large grafts. All allograft reactions became bilateral in the same manner as control grafts of equal-size colonies. Very small pieces of *Montipora* seem more vulnerable to stress and disease in suboptimal conditions.

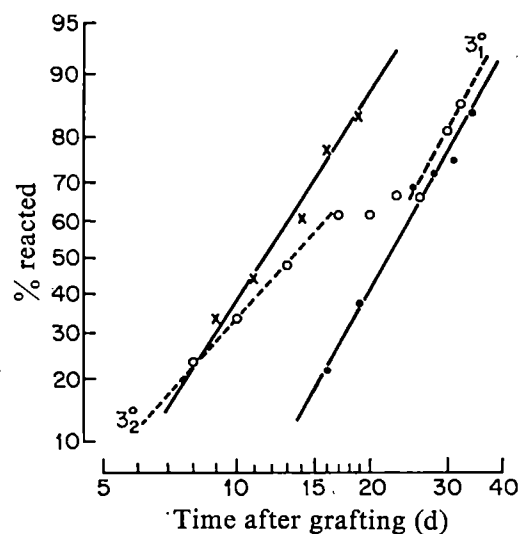
### Induction and duration of alloimmune memory

The occurrence of specific short-term memory was demonstrated by the second-set and third-party reactions evoked when appropriate repeat grafts were placed soon after initial graft pairs had responded incompatibly (Table 1). The questions arise: how long does such positive memory persist and how long does it take to induce such memory? The duration of alloimmune memory was tested

by second-set grafting pairs of corals at new interfaces at 2, 4, 8 and 16 weeks after separation from primary allografting. A 28-d period of primary contact was chosen to assure full sensitisation, since cytotoxic reactions were then underway in all graft pairs. Strong alloimmune memory was present in the second-set group grafted two weeks after first-set separation as shown by an early MRT of 10.7 (8.6–13.4) d (Table 3). This heightened reactivity had faded considerably in *Montipora* test-grafted after 4 weeks. At 8 weeks after first-set separation, specific memory was no longer detectible as evidenced by a MRT of 21.8 (16.1–29.4) d, quite similar to that of control first-set grafts. No further change was demonstrable after 16 weeks.

Possible early induction of memory was tested by disjoining initial allografts after 1, 2, 4 or 8 d of contact and regrafting the same pairs at new interfaces 3 d later. Eight coral pairs regrafted after only 1 d of allogeneic

Fig. 3 Logarithmic probability plots of first-set (●), second-set (×) and third-party (○) allograft reaction times of *M. verrucosa*. MRT difference between 1° and 2° grafts (22.0 and 11.6 d respectively) was highly significant; parallel slopes suggest same cytotoxic events occurred, but at different rates. Unrelated 3° allografts in lieu of specific 2° grafts displayed biphasic distribution of primary-type (3<sub>1</sub>°) and secondary-type (3<sub>2</sub>°) rejections.





**Table 2** Tests for influence of allograft size on cytotoxic reaction times in *M. verrucosa*

Allograft sizes*	No. coral pairs tested	Median reaction times (MRT) (d)†	Range of individual cytotoxic reaction times (d)
(1) 18 × 9 cm <sup>2</sup> (2 : 1)	8	18.2 (13.9–23.9) (7.3)	14–39
(2) 36 × 9 cm <sup>2</sup> (4 : 1)	8	20.9 (16.3–26.7) (7.8)	11–47
(3) 72 × 9 cm <sup>2</sup> (8 : 1)	8	24.4 (19.1–31.3) (9.1)	11–47

Graft dosage or size had little or no effect on the direction or timing of allograft reactivity.

\*Tissue mass ratios shown in parenthesis. Because the smaller piece was the same size (9 cm<sup>2</sup>) in all three experiments, the amount of allogeneic tissue in interfacial contact was also the same.

†Ranges in parentheses are 95% confidence limits; single numbers in parentheses below each entry are the standard deviations of the MRTs. Initial cytotoxic reactions were discernible with equal frequency on small or large grafts; all allograft reactions became bilateral in the same manner as control grafts of equal-size pairs.

contact yielded a MRT of 18.6 (15.5–21.6) d with a range of individual cytotoxic reaction times of 17–45 d. Similar results were obtained in three additional experiments with short-term presensitisation attempted for 2, 4 or 8 d. Individual cytotoxic reaction times ranged from 9–35 d, but only one test-allograft in each of these experiments exhibited an early reaction suggestive of induced immunity. Reaction time ratio tests showed that none of the four MRT's obtained was significantly different from the control first-set value of 22.0 d. Thus little or no pre-immunisation was conferred by short-term allogeneic contact in marked contrast to the potent immunity that resulted from contact for 28 d or longer. These results suggest that systemic immunisation requires longer than 8 d of cell-surface contact. Alternatively, any early immune memory induced seems to fade rapidly.

### Implications of immunoreactivity in lower invertebrates

In the light of earlier studies of advanced invertebrates, we were not surprised to find highly discriminating transplantation specificity in corals coupled to extensive polymorphism of allogeneic H markers probably abundant on cell surfaces. The decisive experiments were observable in nature, whereas our challenge was to quantitate the reactions in controlled laboratory conditions. The finding of specific alloimmune memory in coelenterates was

**Table 3** Duration of alloimmune memory in *M. verrucosa* after separation from primary allografting at 4 weeks

Time of second-set grafting (weeks after 1° separation)	No. coral pairs tested	Median reaction times (MRT) (d)*	Range of individual cytotoxic reaction times (d)
2	7	10.7 (8.6–13.4) (3.4)	9–17
4	8	15.3 (11.4–20.6) (6.8)	10–34
8	7	21.8 (16.1–29.4) (9.3)	12–38
16	7	20.1 (14.6–27.8) (9.1)	12–35

Strong alloimmune memory was present at 2 weeks, but this memory faded considerably by 4 weeks and was completely gone by 8 weeks after primary separation.

\*Ranges in parentheses are 95% confidence limits; single numbers in parentheses below each entry are the standard deviations of the MRTs.

unexpected, however, even though we realised that memory itself might well be an evolving characteristic in multicellular animals<sup>19</sup>. This supposition is now strengthened by the repeated demonstration of short-term memory in *Montipora* in this study. Further investigation of conditions that might favour long-term memory is desirable, because the absence of this characteristic could be a major distinguishing feature of lower metazoans.

The histopathological changes accompanying allocytotoxicity in *Montipora* have proved more difficult to interpret because the diversity of leukocytes or inflammatory cells associated with allograft reactions in higher animals is not evident in coelenterates. Moreover, appropriate techniques for fixation, decalcification and staining of coral tissues are not fully developed. Although we have many histological sections of *Montipora* allograft reaction zones at successive stages of incompatibility, cytotoxicity is obviously reflected only in tissue disruption, localised loss of cells in the immediate contact zone, and excessive mucus secretion. No lymphocytic or phagocytic-type cells, as found to infiltrate allografts in more advanced invertebrates<sup>3–5</sup>, have been detected thus far in our coral grafts. Although coelenterates including corals are endowed with leukocyte-type cells called amoebocytes<sup>20</sup>, their participation in allograft rejection reactions remains conjectural.

Elsewhere a two-component system of (1) specific H marker immunorecognition leading to (2) nonspecific cytotoxic reactions mediated by non-antibody proteins has been suggested as the essential basis for immunocompetence in multicellular invertebrates<sup>21</sup>. The specific induction phase is apparently contingent on polymorphic H receptors leading to activation or triggering of a nonspecific effector phase served by macromolecules with broadly toxic properties. Although specific cell-surface immunorecognition is characteristic of the solitary coral *Fungia*, the non-dialysable, xenocytotoxic molecule(s) secreted in response are nonspecifically reactive with diverse target cells<sup>11,17</sup>. This early but efficient form of immunoreactivity seems to have been retained during progressive evolution of immunocytes and diversification of functions.

The immunoglobulin (Ig) system of antibody-mediated immunity first appears at the level of chordates or fishes as an addition to the postulated H system of cell-mediated immunity already well-developed among invertebrates. Cooperation among lymphocytes and macrophages apparently led to progressive integration of the H and Ig systems eventuating in the immunoregulatory network characteristic of higher vertebrates<sup>21,22</sup>. The heterogeneity of classes of thymus-derived T lymphocytes serving numerous functions among vertebrates, including transplantation immunity, may involve distinctive H and Ig receptors<sup>23</sup>. Possibly the Ig molecules of vertebrates evolved from structurally similar H molecules<sup>24,25</sup> to provide physiologically complex animals with two immunorecognition systems.

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- Cooper, E. L. *J. exp. Zool.* **171**, 69–74 (1969).
- Duprat, F. *Transplant. Proc.* **2**, 222–225 (1970).
- Hildemann, W. H. & Dix, T. G. *Transplantation* **15**, 624–633 (1972).
- Karp, R. D. & Hildemann, W. H. *Transplantation* **22**, 434–439 (1976).
- Reddy, A. L., Bryan, B. & Hildemann, W. H. *Immunogenetics* **1**, 584–590 (1975).
- Tanaka, K. in *Immunologic Phylogeny* (eds Hildemann, W. H. & Benedict, A. A. 115–124 (Plenum, New York, 1975).
- Ivker, F. B. *Biol. Bull.* **143**, 162–174 (1972).
- Theodor, J. L. *Nature* **227**, 690–692 (1970).
- Francis, L. *Biol. Bull.* **14**, 73–92 (1973).
- Bigger, C. H. in *Coelenterate Ecology and Behavior* 127–136 (Plenum, New York, 1976).
- Hildemann, W. H., Linthicum, D. S. & Vann, D. C. *Immunogenetics* **2**, 269–284 (1975).

12. Raison, R. L., Hull, C. J. & Hildemann, W. H. in *Phylogeny of Thymus and Bone Marrow-Bursa Cells* (eds Wright, R. & Cooper, E.) (North-Holland, Amsterdam, 1976).
13. Van de Vyver, G. *Curr. Top. Dev. Biol.* 10, 123-140 (1975).
14. Burnet, F. M. *Receptors Recognition* 1, 35-38 (1976).
15. Lafferty, K. J. & Talmage, D. W. *Transplant. Proc.* 8, 349-353 (1976).
16. Hostetter, R. K. & Cooper, E. L. *Cell. Immun.* 9, 384-392 (1973).
17. Hildemann, W. H. *et al.* in *Proc. 3rd Int. Coral Reefs Symp.* 537-543 (Rosenstiel School of Medicine and Atmospheric Science, University of Miami, Florida, 1977).
18. Litchfield, J. T., Jr *J. Pharmac. exp. Ther.* 97, 399-408 (1949).

19. Hildemann, W. H. *Nature* 250, 116-120 (1974).
20. Chapman, D. M. in *Coelenterate Biology* (eds Muscatine, L. & Lenhoff, H.) 1-92 (Academic, New York, 1974).
21. Hildemann, W. H. *Immunogenetics* (in the press).
22. Jerne, N. K. *Ann. Immunol. (Inst. Pasteur)* 125C, 373-389 (1974).
23. Warr, G. W., Decker, J. M. & Marchalonis, J. J. *Immun. Commun.* 5, 281-301 (1976).
24. Snell, G. D., Dausset, J. & Nathenson, S. *Histocompatibility* (Academic, New York, 1976).
25. Cunningham, B. A. *et al.* *Cold Spring Harb. Symp. quant. Biol.* 41, 351-362 (1977).

# Spatial configuration of mRNA 5'-terminus

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*Nuclear magnetic resonance investigation has revealed that the 5'-terminus m<sup>7</sup>G<sup>5'</sup>ppp<sup>5'</sup>Am of mRNA displays a spatial configuration in which the bases form stacked arrays. Details of the conformation as derived from coupling constants, shift trends and ring current considerations are discussed.*

THE 5'-terminal regions of a wide variety of mRNAs of eukaryotes contain an unusual sequence of nucleotides in which the terminal is 7-methyl-guanosine<sup>1-11</sup> (Fig. 1). Several studies<sup>10-15</sup> have shown that this sequence for the 5' termini of mRNAs is obligatory to carry out *in vitro* translation with fidelity.

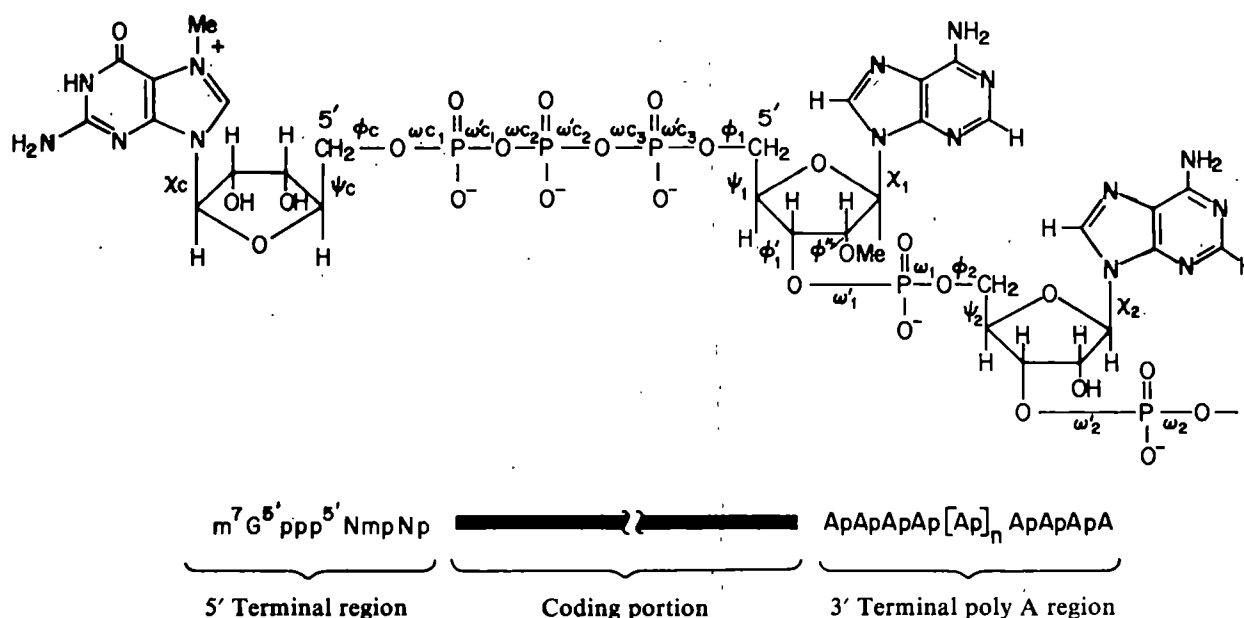
To understand the structure-function relationships we have explored whether the uncommon constitution of the 5' termini endows any unique stereochemical features to mRNAs. The conformational properties of the 5' termini were investigated by studying the solution conformations of: (1), 7-methylated guanosine derivatives compared with the corresponding non-methylated analogues; (2), 2'-O-methylated nucleosides and the corresponding 5'-nucleotides compared with the non-methylated analogues; and (3), the dimer m<sup>7</sup>G<sup>5'</sup>ppp<sup>5'</sup>Am.

All materials used were commercial preparations. <sup>1</sup>H nuclear magnetic resonance (NMR) spectra were recorded at 270 MHz in the Fourier transform mode using systems described elsewhere<sup>16-18</sup>. All NMR spectra were analysed using a UNIVAC 1110 computer and LACON III. Figure 2 illustrates the observed and computer-simulated spectra of m<sup>7</sup>G<sup>5'</sup>ppp<sup>5'</sup>Am at two temperatures. The coupling constants data derived for the various monomers and the dimer were translated into conformational parameters using equations developed by Lee and Sarma<sup>19</sup> and Lee *et al.*<sup>18</sup> and the data are summarised in Table 1.

## Conformational properties of 7-methylated monomers

In general the ribose ring of common nucleosides and nucleotides at the examined pH and temperature exist as <sup>2</sup>E⇌<sup>3</sup>E equilibrium mixture with a bias of ≈ 60% for <sup>2</sup>E conformer (Table 1). Inspection of the ribose conformer populations for the non-methylated and methylated systems clearly reveals that 7-methylation has a decisive influence. The data show that this causes 13-24% increase in <sup>3</sup>E conformers and the increase closely follows the number of phosphate groups introduced. It is important to note that 7-methylated di- and triphosphates tend to populate in <sup>3</sup>E conformation and this is the first observed case in which purine 5'-nucleotides show a preference for <sup>3</sup>E pucker in

**Fig. 1** The sequence of mRNA at the 5'-region. The above sequence can be abbreviated as m<sup>7</sup>G<sup>5'</sup>ppp<sup>5'</sup>AmpA. Structures in which AmpA has been replaced with any of the common bases have been observed in mRNA. The conformational nomenclature  $\chi$ ,  $\psi$ ,  $\phi$ ,  $\omega$ ,  $\phi'$ ,  $\phi''$  and so on follow definitions according to IUPAC-IUB. In the present paper a letter 'c' is inserted to distinguish between the cap region and the AmpA region. Thus  $\chi_c$  stands for sugar base torsion of m<sup>7</sup>Gp- and  $\chi_1$  and  $\chi_2$  for the Amp- and -pA parts respectively. Non-standard abbreviations such as G<sup>5'</sup>p, G<sup>5'</sup>pp are used for 5'-GMP, 5'-GDP and so on in accordance with their wide use in the original papers<sup>1-11</sup>.



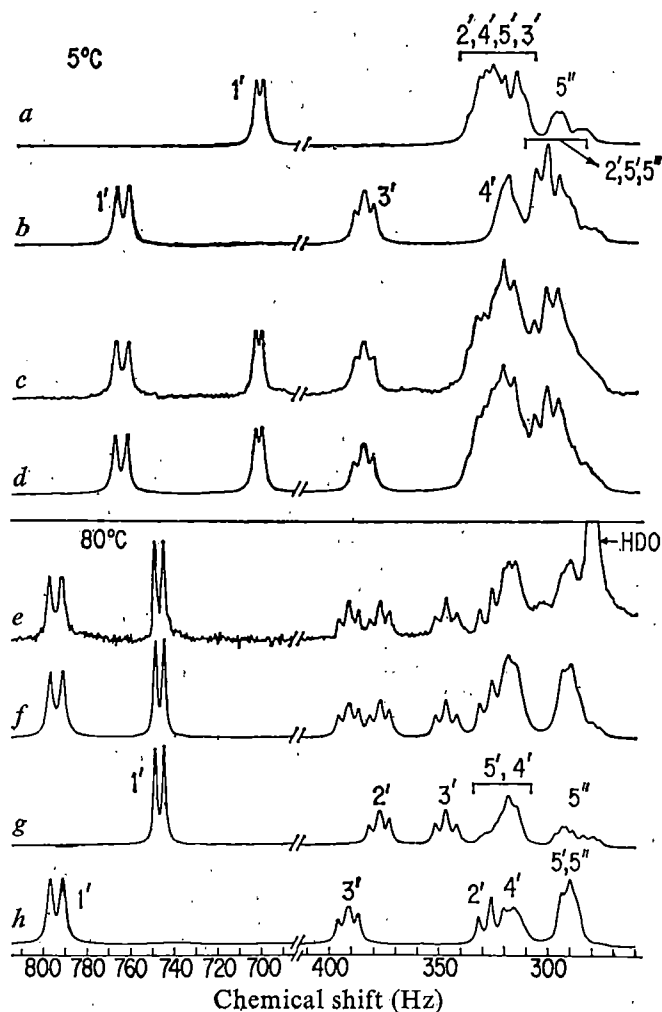


Fig. 2 The observed 270-MHz  $^1\text{H}$  NMR spectra of  $m^7\text{G}^{5'}\text{ppp}^{5'}\text{Am}$  at  $5^\circ\text{C}$  (c) and  $80^\circ\text{C}$  (e). Both spectra were analysed by computer simulation of  $m^7\text{G}^{5'}\text{p}$ - (a and g) and  $-p^{5'}\text{Am}$  parts (b and h) separately (a and b at  $5^\circ\text{C}$  and g and h at  $80^\circ\text{C}$ ) and combining them to produce complete simulation at  $5^\circ\text{C}$  (d) and at  $80^\circ\text{C}$  (f). The chemical shifts are Hz relative to tetramethylammonium chloride.

aqueous solution. In the 7-methylinosine series 5'-phosphorylation has no effect on ribose conformation, in the contrast to the 7-methylguanosine series. Changing the ionisation state of the phosphate causes only a slight shift to  $^3\text{E}$  pucker for  $m^7\text{G}^{5'}\text{p}$ . In the case of di- and triphosphate this has an opposite effect, that is,  $^2\text{E}$  population increases. With respect to the common derivatives only the diphosphates are sensitive to ionisation changes.

The data indicate that irrespective of whether mono-, di- or triphosphate, or whether 7-methylated or not, all nucleotides show an overwhelming preference for  $g'g'$  orientation ( $\phi_c = 180^\circ$ ) about  $\text{C}5'-\text{O}5'$ . There is, however, a drastic change on conformational preference about  $\text{C}4'-\text{C}5'$  in going from 7-methylated nucleoside to corresponding nucleotide. The population of  $gg$  orientation ( $\psi_c = 60^\circ$ ) significantly increases on 5'-mono-, di- or triphosphorylation of 7-methylguanosine. A possible reason for this overwhelming increases in  $gg$  population is the electrostatic interaction between the positively charged N7 and the negatively charged phosphate groups. In the  $gg$  conformation the distance between the phosphate oxygens and N7 is minimum compared with that in the alternate  $gt$  ( $\psi_c = 180^\circ$ ) and  $tg$  ( $\psi_c = 300^\circ$ ) conformers. The phosphorus chemical shift data summarised in Table 2 clearly substantiate this electrostatic interaction. Particularly noteworthy is the substantial difference in chemical shifts between the  $\gamma$  phosphorus of  $m^7\text{G}^{5'}\text{ppp}$  and  $\text{G}^{5'}\text{ppp}$ .

It should be noted that  $m^7\text{G}^{5'}\text{p}$ ,  $m^7\text{G}^{5'}\text{pp}$  and  $m^7\text{G}^{5'}\text{ppp}$  exist

with almost exclusive preference for the  $gg$  orientation about  $\text{C}4'-\text{C}5'$ . This presents a drastic contrast to the situation in  $\text{G}^{5'}\text{p}$ ,  $\text{G}^{5'}\text{pp}$  and  $\text{G}^{5'}\text{ppp}$ , all of which exist as a conformational blend in which the  $gg$  population range from 67–77%, the remaining being the alternate  $gt$  and  $tg$  conformers. This is the first time a nucleotide in aqueous solution has been shown to exist with a rigid geometry about  $\text{C}4'-\text{C}5'$ . The data thus point out the impact of 7-methylation on the conformational freedom about  $\text{C}4'-\text{C}5'$  bond in the guanosine 5'-nucleotides. This is a general phenomenon associated with 5'-purine nucleotides and not something endemic to the guanosine system is illustrated by the data on  $m^7\text{I}$  and  $m^7\text{I}^{5'}\text{p}$  (Table 1).

X-ray and NMR data and theoretical calculation on mono- and dinucleoside monophosphates (see ref. 18 and refs therein), have shown that the preferred glycosidic torsion in these systems lie in the anti domain. In the 7-methylguanosine series the anti domain is congenial for the electrostatic interaction discussed above.

### Conformational properties of 2'-O-methylated monomers

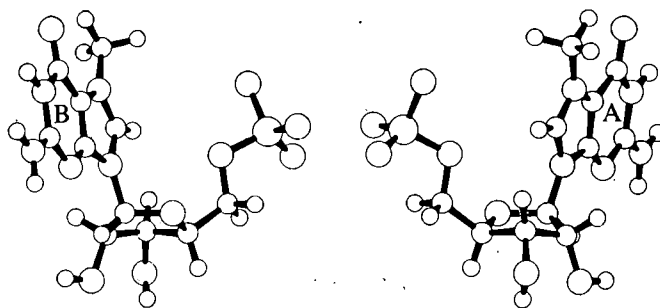
The data in Table 1 for the 2'-O-methylated nucleosides and nucleotides reveal that at the monomer level 2'-O-methylation has only very little influence on the conformational preferences of the ribose ring,  $\text{C}4'-\text{C}5'$  and  $\text{C}5'-\text{O}5'$  bonds. They, in general, exist as a conformational blend in which  $^2\text{E}$  pucker,  $gg$  and  $g'g'$  conformations are preferred. The influence of the ionisation of phosphate on the chemical shifts of base and ribose protons (data not given) reveal that in this system the base occupies anti domain.

### Conformational properties of the dimer $m^7\text{G}^{5'}\text{ppp}^{5'}\text{Am}$

The conformational parameters (Table 1) indicate that  $m^7\text{G}^{5'}\text{p}$ - and  $-p^{5'}\text{Am}$  of the dimer show a significant preference to orient the  $\text{C}4'-\text{C}5'$  and  $\text{C}5'-\text{O}5'$  backbone in the classically staggered conformation in which  $\psi = 60^\circ$ ,  $\phi = 180^\circ$ . But,  $m^7\text{G}^{5'}\text{p}$ - component prefers  $^3\text{E}$  sugar pucker and  $-p^{5'}\text{Am}$  component prefers  $^2\text{E}$  sugar pucker. Comparison of the conformational data for the dimer with that of the monomers at pH 5 (Table 1) clearly indicates that with respect to the local conformations about the sugar ring,  $\text{C}4'-\text{C}5'$  and  $\text{C}5'-\text{O}5'$  bonds the monomers essentially conserve their conformation in the dimer. There is a small but real shift towards  $^3\text{E}$  pucker in the dimer for the  $m^7\text{G}^{5'}\text{p}$ - part, however. This small shift may indicate a reduction in the  $\chi_c$  of the dimer compared with that of the monomer, because of the conformational nexus between ribose pucker and  $\chi_{\text{CN}}$  (ref. 18).

Information about the molecular topology and intramolecular order of the dimer can be obtained from comparison of the chemical shift data for monomers and the dimer. These dimerisation data are presented in Table 3. The data clearly show that the two nucleotidyl units at the 5' ends of the triphosphate bridge interact with each other, changing the effective field felt at the various protons. As a result of this interaction the chemical shifts of the two residues undergo drastic changes. The protons which show negligible changes are only  $\text{H}4'$ ,  $\text{H}5'$  and  $\text{H}5''$  of the  $m^7\text{G}^{5'}\text{p}$ -residue. The  $\text{H}5'$  and  $\text{H}5''$  of the  $-p^{5'}\text{Am}$  residue undergo shifts to

Fig. 3 The two perspectives, drawn using ORTEP, of  $m^7\text{G}^{5'}\text{p}$ . The left one shows the 'B' side of purine and the right one the 'A' side. The ribose is  $^3\text{E}$ ,  $\psi = 60^\circ$ ,  $\phi = 180^\circ$  and  $\chi_c = 60^\circ$ . This is the most preferred conformation of  $m^7\text{G}^{5'}\text{p}$ .



**Table 1** Conformational parameters for various components of 5'-terminus of mRNA and corresponding analogues in D<sub>2</sub>O

Common nucleosides and nucleotides	pH	% conformational preferences about various bonds			
		CS'-OS' g'g'↔g'/t'	C4'-C5' gg↔g/t	Ribose 3'E↔2'E	
G	7.2	—	69 31	38	62
G <sup>5'</sup> p	8	75 25	67 33	36	64
	5	71 29	71 29	37	63
G <sup>5'</sup> pp	7	71 29	69 31	44	56
	5	74 26	71 29	37	63
G <sup>5'</sup> ppp	7	68 32	77 23	36	64
	5	70 30	79 21	37	63
A	8	—	75 25	36	64
p <sup>5'</sup> A	7	76 21	75 25	38	62
	5	72 28	77 23	39	61
pp <sup>5'</sup> A	8	69 31	79 21	47	53
	5	72 28	79 21	40	60
7-methylated derivatives					
m <sup>7</sup> G	7	—	76 24	51	49
m <sup>7</sup> G <sup>5'</sup> p	7	77 23	97 3	54	46
	5	74 26	91 9	60	40
m <sup>7</sup> G <sup>5'</sup> pp	7	76 24	100 0	65	35
	5	78 22	100 0	53	47
m <sup>7</sup> G <sup>5'</sup> ppp	7	75 25	100 0	60	40
	5	76 24	100 0	51	49
m <sup>7</sup> I	7	—	77 23	56	44
m <sup>7</sup> I <sup>5'</sup> p	7	79 21	95 5	57	43
	5	72 28	98 2	58	42
2'-O-methylated derivatives					
Am	7	—	78 22	33	67
p <sup>5'</sup> Am	7	73 27	75 25	35	65
	5	70 30	77 23	38	62
pp <sup>5'</sup> Am	7	73 27	76 24	40	60
	5	72 28	79 21	36	64
Gm	7	—	69 31	37	63
p <sup>5'</sup> Gm	7	74 26	73 27	35	65
pp <sup>5'</sup> Gm	7	69 31	76 24	42	58
ppp <sup>5'</sup> Gm	7	69 31	66 34	35	65
Dimer m <sup>7</sup> G <sup>5'</sup> ppp <sup>5'</sup> Am					
m <sup>7</sup> G <sup>5'</sup> p-	7 (5 °C)	81 19	100 0	61	39
	7 (80 °C)	75 25	90 10	51	49
-p <sup>5'</sup> Am	7 (5 °C)	74 26	88 12	40	60
	7 (80 °C)	72 28	79 21	40	60

Concentration is 0.02–0.07 M for the monomers except for G(0.004 M) and 0.007 M for m<sup>7</sup>G<sup>5'</sup>ppp<sup>5'</sup>Am. The temperature in all cases was 19 ± 1 °C unless otherwise stated.

lower field. The remaining protons of both residues are shifted to higher field by as much as 60 Hz. From the observation that H8 and H2 of the -p<sup>5'</sup>Am moiety and 7-CH<sub>3</sub> group of m<sup>7</sup>G<sup>5'</sup>p- moiety are shifted to higher field it is immediately apparent that the two aromatic systems interact with each other as probably in a parallel stacking.

It should be emphasised that the interaction between bases takes place while the individual nucleotidyl units maintain very similar conformation for ribose, C4'-C5' and CS'-OS' bonds as in the monomers. Hence this intramolecular stacking interaction must be made feasible by torsional variation around the phosphodiester bonds of the triphosphate bridge. To describe the stacking possibilities between the two ring systems it is necessary to label the sides of purine moieties by the letters A and B as has been done before<sup>20–22</sup>. These are shown in Fig. 3 for m<sup>7</sup>G<sup>5'</sup>p-. There are four possible types of stacking: (1) A-A stacking. A surface of m<sup>7</sup>G<sup>5'</sup>p- moiety faces the A surface of -p<sup>5'</sup>Am moiety (Fig. 4a). (2) A-B stacking. A surface of m<sup>7</sup>G<sup>5'</sup>p- moiety faces the B surface of -p<sup>5'</sup>Am moiety (Fig. 4b). (3) B-A stacking. B surface of m<sup>7</sup>G<sup>5'</sup>p- moiety faces the A surface of -p<sup>5'</sup>Am moiety (Fig. 4c). (4) B-B stacking. B surface of m<sup>7</sup>G<sup>5'</sup>p- moiety faces the B surface of -p<sup>5'</sup>Am moiety (Fig. 4d).

In A-A and B-B stacking the O1' of both ribose rings are oriented in opposite direction whereas in A-B and B-A stacking they are oriented in the same direction (Fig. 4). The four stacking arrangements depicted in Fig. 4 are generated while maintaining the same conformation for the ribose rings, C4'-C5' and CS'-OS'

bonds, and the only changes are in  $\chi_c$ ,  $\chi_1$ ,  $\omega C_1$ ,  $\omega' C_1$ ,  $\omega C_2$ ,  $\omega' C_2$ ,  $\omega C_3$  and  $\omega' C_3$ .

The observation that the base protons of -p<sup>5'</sup>Am moiety and 7-methyl group of m<sup>7</sup>G<sup>5'</sup>p- moiety are shielded (Table 3) can be rationalised on the basis of any of the above stacked structures or on the basis of a blend of all the four forms. One should, however, be able to make a distinction by following the shift trends of interior protons such as H1', H2', H3', H4', H5', and H5". The shifts of H5' and H5" could be affected by torsional changes in the phosphate backbone as a dimer is formed from the monomer, and hence are of little use in the determination of stacking interactions from ring current considerations. The H1' chemical shift of both residues will be highly sensitive to  $\chi_{CN}$  variation and the observed upfield shift of H1' (Table 3) is an indication of reduction in  $\chi_{CN}$ .

**Table 2** Chemical Shift data (Hz) of phosphorus in guanosine derivatives

Compounds	$\alpha$	$\beta$	$\gamma$
G <sup>5'</sup> pp	556	410	—
m <sup>7</sup> G <sup>5'</sup> pp	566	428	—
G <sup>5'</sup> ppp	566	999	400
m <sup>7</sup> G <sup>5'</sup> ppp	576	1014	423

Shifts are given relative to internal trimethylphosphate, (CH<sub>3</sub>O)<sub>3</sub>OP. Concentration is 0.05 M, pH 7.0 at 19 ± 1 °C. 40.5 MHz NMR system.



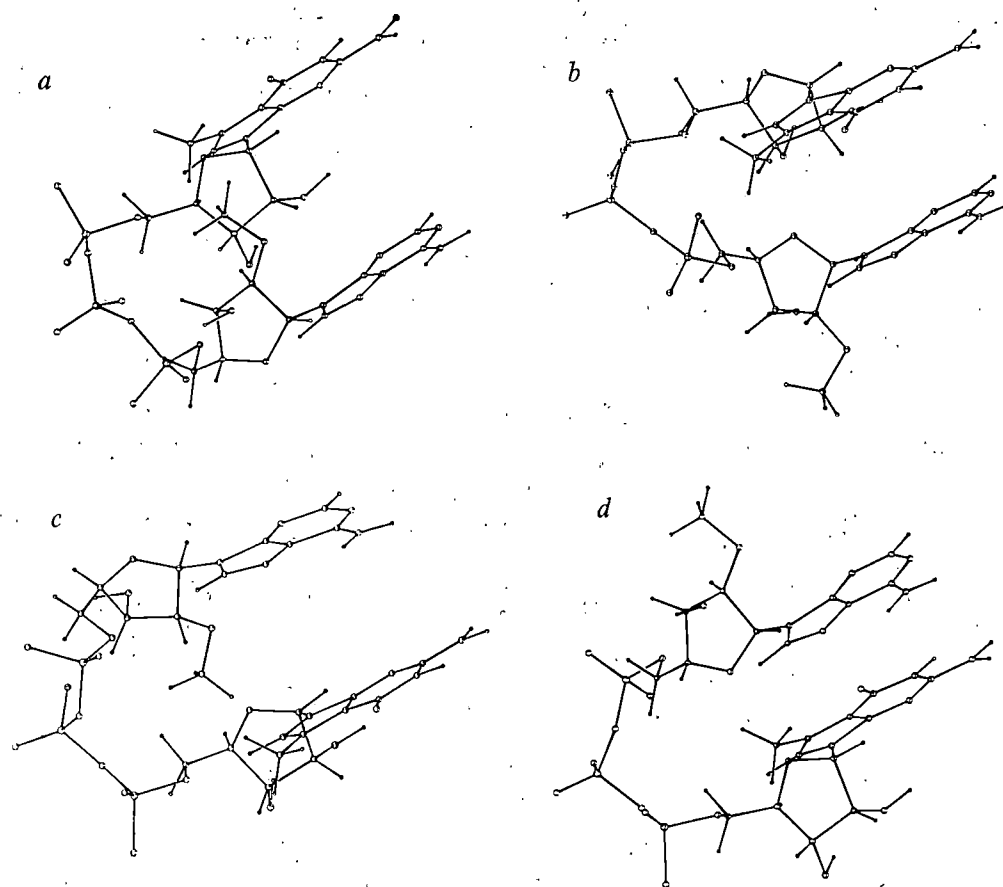


Fig. 4 The four basic stacked arrays A-A (a), A-B (b), B-A (c) and B-B (d) possible for  $m^7G^{5'}ppp^{5'}Am$ . The perspectives were generated using ORTEP. In all the projections the ribose of the  $m^7G^{5'}p$ - part is  $^3E$  and that of the  $-p^{5'}Am$  part is  $^2E$ , further  $\psi_c = 60^\circ$ ,  $\phi_c = 180^\circ$ ,  $\phi_1 = 180^\circ$ ,  $\psi_1 = 60^\circ$  and  $\phi'' = 180^\circ$ . These are the experimentally observed preferred values. The values for  $\chi_c$  and  $\chi_1$ , as well as those for  $\omega C1/\omega' C1$ ,  $\omega C2/\omega' C2$ ,  $\omega C3/\omega' C3$  cannot be obtained directly from NMR data because of the lack of appropriate nuclei to produce the desired coupling constants. But the data clearly reveal that the bases are in the antidiagonal and the orientation of the phosphodiester bonds allow stacking interactions. A search in the conformation space produced a set of values for  $\chi_c$ ,  $\chi_1$ ,  $\omega C1/\omega' C1$ ,  $\omega C2/\omega' C2$  and  $\omega C3/\omega' C3$  which can generate the four possible stackings.

(refs 23, 24) on dimerisation. This is anticipated because dimerisation and stacking will necessitate the readjustment of  $\chi_{CN}$  in the dimer compared to the values in the monomers. It should be noted that in both dimer and monomers the base prefers anti domain but the magnitude of  $\chi_{CN}$  in the dimer is smaller than that in the monomer. NMR methodology cannot precisely determine the magnitude of  $\chi_{CN}$  but can only provide relative changes<sup>24</sup>. If the value of  $\chi_{CN}$  in the monomer is between  $50^\circ$  and  $60^\circ$ , the value in the dimer will be between  $30^\circ$  and  $40^\circ$ . Hence, one cannot use the shift trends of  $H1'$  to monitor the stacking interactions. But, the observation that  $H2'$  and  $H3'$  of  $m^7G^{5'}p$ - and  $-p^{5'}Am$  have undergone significant shielding to higher fields can be rationalised only on the basis of the presence of significant populations of A-A stacking. Inspection of perspectives (Fig. 4a-d) clearly reveals that only in an A-A stack these four protons lie inside of the stack. Thus  $H2'$  (see Table 3) and  $H3'$  of  $m^7G^{5'}p$ - moiety can be shielded

by the adenine of  $-p^{5'}Am$ , conversely  $H2'$  and  $H3'$  of  $-p^{5'}Am$  moiety can be shielded by the guanine ring of  $m^7G^{5'}p$ -. This mutual shielding is possible only in an A-A stack. The observed difference in the magnitude of shielding between the sets of  $H2'$  and  $H3'$  protons of two moieties is merely a reflection of the difference in the ring current fields of adenine and guanine moieties<sup>26</sup>. The ring current shielding abilities of guanine are less than that of adenine<sup>25,26</sup>. In the present case the guanine ring contains positive charge and this will further dilute the effect of guanine. The data in Table 3 clearly show that the adenine shields the  $H2'$  and  $H3'$  of  $m^7G^{5'}p$ - considerably more effectively than guanine shields the  $H2'$  and  $H3'$  of  $-p^{5'}Am$ . This is indeed expected.

Because of the inherent flexibility of the various phosphodiester linkages, in aqueous solution it may be that the dimer  $m^7G^{5'}ppp^{5'}Am$  exists as a conformational blend of the various stacked arrays and extended forms. Our major conclusion is that among the

Table 3 Effects of dimerisation and temperature change on chemical shift

	Dimerisation effect* (Hz)		Temperature effect† (Hz)	
	$m^7G^{5'}p$ -	$-p^{5'}Am$	$m^7G^{5'}p$ -	$-p^{5'}Am$
AH8	—‡	27.6	—‡	18.7
AH2	—	22.8	—	36.1
2'-O-Methyl	—	11.8	—	9.2
7-Methyl	25.7	—	22.8	—
H1'	62.6	39.8	44.5	29.0
H2'§	60.6	46.3	43.3	24.5
H3'	46.3	28.1	29.6	6.0
H4'	1.5	6.9	-10.0	-5.7
H5'	1.7	-18.0	2.0	-15.5
H5''	-0.7	-11.5	-4.5	-1.0

\*Value in monomer minus that in dimer.

†Value for dimer at  $80^\circ C$  minus that for dimer at  $5^\circ C$ .

‡GH8 exchanges with  $D_2O$  and could not be located.

Note that even though the data indicate significant high field shift for  $H2'$ , the theoretically projected<sup>25,26</sup> shielding of  $H2'$  originating from stacking interactions in A-A stacks should be larger than what is observed. This is because, this high field shift is internally compensated by the down-field shift<sup>23</sup> of  $H2'$  caused by reduction in  $\chi_c$  associated with  $^3E$  sugar pucker.

various conformational possibilities in aqueous solution the A-A stack predominates and the molecule has an inherent proclivity to form A-A stacked arrays. The temperature data on  $m^7G^{5'}ppp^{5'}Am$  summarised in Table 3 closely parallel the dimerisation trends and these observations are essentially in agreement with the findings of Ts'o and coworkers<sup>27</sup> that elevation of temperature causes de-stacking and at higher temperatures the dimer tends to approximate to monomer conformations.

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1. Rottman, F., Shatkin, A. J. & Perry, R. P. *Cell* **3**, 197-199 (1974).
2. Furuichi, Y. & Miura, K.-I. *Nature* **253**, 374-375 (1975).
3. Furuichi, Y., Morgan, M., Muthukrishnan, S. & Shatkin, A. J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 362-366 (1975).
4. Furuichi, Y., Muthukrishnan, S. & Shatkin, A. J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 742-745 (1975).

5. Urushibara, T., Furuichi, Y., Nishimura, C. & Miura, K.-I. *FEBS Lett.* **49**, 385-389 (1975).
6. Wei, C. M. & Moss, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 318-322 (1975).
7. Abraham, G., Rhodes, D. P. & Banerjee, A. K. *Nature* **255**, 37-40 (1975).
8. Muthukrishnan, S., Both, G. W., Furuichi, Y. & Shatkin, A. J. *Nature* **255**, 33-37 (1975).
9. Furuichi, Y. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 1904-1908 (1975).
10. Adams, J. & Cary, S. *Nature* **255**, 28-33 (1975).
11. Furuichi, Y., LaFiandra, A. & Shatkin, A. J. *Nature* **266**, 235-241 (1977).
12. Muthukrishnan, S., Morgan, M., Banerjee, A. K. & Shatkin, A. J. *Biochemistry* **15**, 5761-5768 (1976).
13. Shafritz, D. *et al. Nature* **261**, 291-294 (1976).
14. Hickey, E. D., Weber, L. A. & Baglioni, C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 19-23 (1976).
15. Hickey, E. D., Weber, L. A., Baglioni, C., Kim, C. H. & Sarma, R. H. *J. molec. Biol.* **109**, 173-183 (1977).
16. Singh, H., Herbut, M. H., Lee, C. H. & Sarma, R. H. *Biopolymers* **15**, 2167-2184 (1976).
17. Lee, C. H. & Sarma, R. H. *Biochemistry* **15**, 697-704 (1976).
18. Lee, C. H., Ezra, F. S., Kondo, N. S., Sarma, R. H. & Danyluk, S. S. *Biochemistry* **15**, 3627-3639 (1976).
19. Lee, C. H. & Sarma, R. H. *J. Am. chem. Soc.* **98**, 3541-3548 (1976).
20. Sarma, R. H. & Mynott, R. J. *J. Am. chem. Soc.* **95**, 7470-7480 (1973).
21. Sarma, R. H. & Mynott, R. J. *Conform. Biol. Molec. Polym., Proc. Jerusalem Chem. Biochem. S.* **5**, 591-626 (1973).
22. Evans, F. E. & Sarma, R. H. *Biopolymers* **13**, 2117-2132 (1974).
23. Giessner-Pretre, C. & Pullman, B. *J. theor. Biol.* **65**, 189-201 (1977).
24. Giessner-Pretre, C. & Pullman, B. *J. theor. Biol.* **65**, 171-188 (1977).
25. Giessner-Pretre, C., Pullman, B., Borer, P. N., Kan, L.-S. & Ts'o, P. O. P. *Biopolymers* **15**, 2277-2286 (1976).
26. Giessner-Pretre, C. & Pullman, B. *J. theor. Biol.* **27**, 87-95 (1970).
27. Ts'o, P. O. P., Kondo, N. S., Schweizer, M. P. & Hollis, D. P. *Biochemistry* **8**, 997-1029 (1969).

# letters to nature

## Evidence for a 39-d period in Cyg X-1

THE detection of a 39-d period in Cyg X-1 (HDE226868), as seen in the ultraviolet (ultraviolet-filter) polarisation<sup>1</sup>, would have at least one interesting interpretation, that of a third-body orbital period (M. Milgrom and J. Shaham, personal communication). We report here the result of long-term monitoring of the optical polarisation of Cyg X-1<sup>2</sup>, which has been in progress since 1974 at Pine Mountain Observatory, Oregon. Up to early August 1977, over 225 nights of ultraviolet-filter data were obtained. Power spectra of the ultraviolet-filter polarisation computed in March 1977 suggested a peak at the approximate period 39 d, common to the separate power spectra of our two instrumental Stokes parameters<sup>2</sup>  $Q$  and  $U$ . The amplitude of the feature corresponds to a peak-to-peak polarisation variation of about 0.25%. The  $Q$  and  $U$  variations proved to be coherent with each other and to be of a rectilinear type, describable as a simple variation along a certain rotated Stokes parameter  $Q_0$ , defined approximately by  $Q_0 = p \cos[2(\theta - 117^\circ)]$ . The normalised conjugate parameter  $U_0 = p \sin[2(\theta - 117^\circ)]$  shows no significant variation at the 39-d period.

Cyg X-1 has an interstellar polarisation of roughly 5%, on which variations of the order of  $\pm 0.3\%$  are superimposed. The variability is partly or largely random in character; the isolation of periodicities, therefore, requires a long-term programme. We have also recorded a slow secular change in  $p$ , the degree of polarisation, averaging about  $0.00047\% \text{ d}^{-1}$  in the ultraviolet; this has amounted to a shift from an average  $p = 4.25\%$  in 1974 to  $4.55\%$  in 1977.

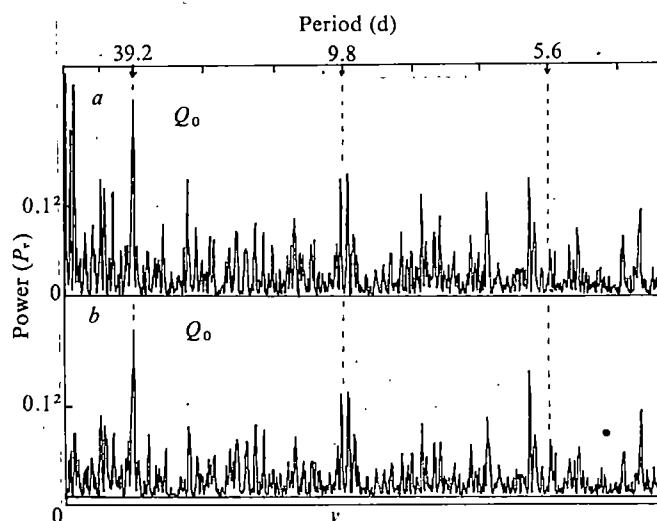
Figures 1 and 2 show power spectra of the ultraviolet-filter polarisation, computed by the bin method<sup>2</sup>, using data from 192 nights spanning the interval September 1974-June 1977. The two spectra in Fig. 1 are of the  $Q_0$  parameter, in which the 39-d effect is most evident. Figure 2 shows the power spectra of  $Q_0$  and  $U_0$  using the slope-corrected data after a mild weighting, in which all data entries (nights) which exhibit excursions  $\Delta Q$  or  $\Delta U$  exceeding 0.7% have been assigned relative weights of 0.5 (15 nights are thus de-weighted).

The 39-d peak in  $Q_0$  is the highest peak in all of our power spectra, apart from very long-period structure ( $P \geq 400 \text{ d}$ ) when the secular slope is not removed as in Fig. 1a. In later results, using 225 nights of data, the contrast of the 39-d feature has improved. A very rapid improvement cannot be expected.

Statistical aspects of the apparent 39.2-d periodicity are summarised in Table 1, which is based on an analysis made with a

packaged statistical program. The latter performs linear regression and yields various data such as  $F$  values. We used a multi-function fit to the raw data including: (1) a slope term, to account for the secular change in polarisation; (2) the predominant 39.2-d sinusoid; and sometimes (3) a harmonic, of period  $39.2 \text{ d}/n$ , where  $n$  is an integer. (The case  $n=0.5$  is also included in Table 1.) The likelihood of a null hypothesis for each frequency component, of arbitrary phase but pre-assigned period, is given in column 3. Those probabilities must be multiplied by the number of statistically-independent periods, that is by  $N/2$  where in this case

Fig. 1 Power spectra of Cyg X-1 ultraviolet-filter polarisation, based on 192 nights from September 1974 to mid-June 1977. (One data point per night is taken, involving 1-3 h of integration including calibrations.) Computed by simple bin-averaging method of Kemp *et al.*<sup>2</sup>. Such power spectra have slightly poorer signal-to-noise ratios than spectra computed from linear regression, but are less expensive in computer time. Scan uses 1,000 discrete frequencies, six bins. *a*, raw data; *b*, computed from data with secular slope ( $\Delta p = +0.0005\% \text{ d}^{-1}$ ) removed thus suppressing the structure at very long periods ( $> 200 \text{ d}$ ). Parameter  $Q_0$  has maximum amplitude at 39.2 d. In all the figures, ordinate is proportional to power, that is to the squared Fourier amplitudes of the polarisation in per cent; abscissa is linear in frequency (inverse period).



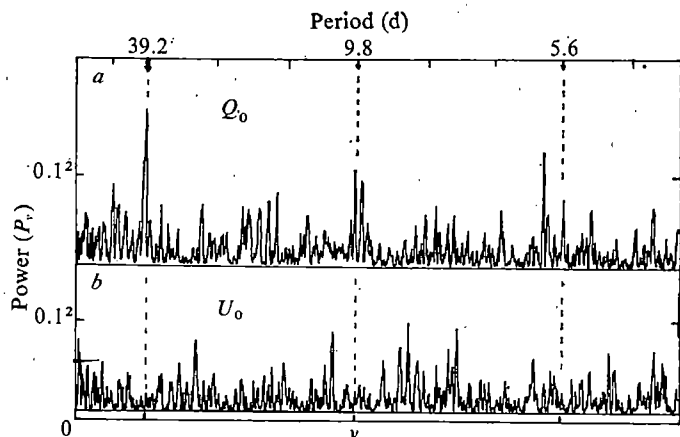


Fig. 2 Power spectra with slope removed, and with data slightly weighted; 19 points with  $a, \Delta Q$  or  $b, \Delta U$  (variations from means) given weights 0.5, all others given weights unity. A very slight improvement is noticed in the apparent signal-to-noise ratio. Both Stokes parameters are shown.

$N=192$ . We must also multiply by 2, to account for the two Stokes parameters  $Q_0$  and  $U_0$ . Unbiased probabilities for a random-noise hypothesis are thus given in column 4.

From this analysis, and other criteria, we have concluded that the 39.2-d periodicity is real with a probability which now exceeds 97%.

Of the harmonics, only the fourth (of period 9.8 d) seems to be appreciable. In Table 1 the low amplitudes at 2.8 d and 5.6 d, are of special interest the latter being the well-known binary orbital period.

Figure 3 shows an amplified trace of the  $Q_0$  power spectrum in the vicinity of 39 d. A least-mean-squares fit to the function  $(A \sin \Delta v / \Delta v)^2$  has been made. We find a best-fit period of 39.22 d for the bin-type power spectrum, and 39.30 d for the linear-regression points—or an average of about 39.26 d. To three significant figures<sup>3</sup> the binary period of Cyg X-1 is 5.60 d. We thus contemplate the ratio  $39.26/5.60 = 7.01$ . That is, the 39.2-d period is very likely the exact seventh multiple of the binary period.

Because of the almost integral relationship just shown, one might suspect that the 39-d pattern is an aliasing effect of some kind, but this is unlikely. The test power spectra obtained by applying pure sinusoids to our sampling times have negligible side lobes, less than 1/5 as strong as the central peaks, as we have tested for 39.2-d, 5.6-d and 2.8-d fundamental periods; and furthermore, the Fourier amplitudes at 5.6 and 2.8 d in the observed power spectra are very weak. A confusion with aliases having periods very close to 1.0 d, namely the periods  $(1.0 \text{ d} \pm 1/39.2 \text{ d})$ , cannot be

ruled out, because our observations are always separated by about 1 d or more. We discount that possibility.

As yet the 39-d periodicity has not been detected in other properties. A search of the X-ray flux records does not show such a period (S. S. Holt, and E. N. Walker, personal communications); but the X-ray variability is known to have at best only a tenuous connection with the optical behaviour<sup>4</sup>. Virtually all of the optical photometry of Cyg X-1 has been performed in blue filter (blue light). There is no indication of a 39-d period in the blue photometry (E. N. Walker, and I. G. Nolt personal communications). On the other hand, it is likely that the 39-d effect is

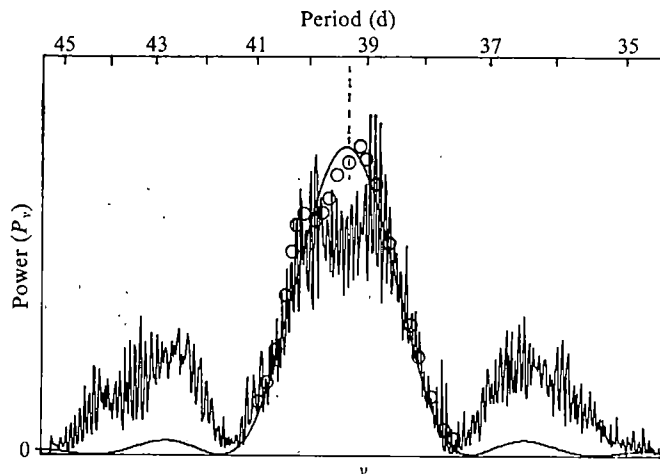


Fig. 3 Local power spectrum,  $Q_0$  parameter, around 39-d period. Jagged curve<sup>2</sup> is generated by bin-analysis program; circle points were obtained one at a time from multiple regression program including a slope plus sine and cosine (for each period). A curve fit to the diffraction-like function is shown.

wavelength-dependent and is absent in the blue-visible: the ultraviolet-blue-visible polarimetry of 1975<sup>2</sup> indicate  $Q_0$  amplitudes of  $\leq 0.06\%$  in blue and  $\leq 0.08\%$  in visible. Ultraviolet photometry, currently under investigation at our observatory, may answer the question of a photometric counterpart of the polarisation period.

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1. Kemp, J. C. *I.A.U. Circ.* No. 3060 (1977).
2. Kemp, J. C., Southwick, R. G. & Rudy, R. J. *Astrophys. J.* **210**, 239-249 (1976).
3. Bolton, C. T. *Astrophys. J.* **200**, 269-277 (1975).
4. Holt, S. S., Boldt, E. A., Serlemitsos, P. J. & Kaluzienski, L. J. *Astrophys. J. Lett.* **203**, L63-L66 (1976).

Table 1 Cyg X-1, ultraviolet polarisation; components of 39.2-d variation and harmonics from linear regression

Period (d)	Amplitude (in % polarisation)	Simple null probability*	Unbiased null probability†
<b><math>Q_0</math> Stokes parameter</b>			
39.2‡	$0.129 \pm 0.032$	$1.1 \times 10^{-4}$	0.021
39.2§	$0.131 \pm 0.030$	$0.7 \times 10^{-4}$	0.013
9.8§	$0.087 \pm 0.031$	0.0036	(0.69)
5.6	$0.041 \pm 0.031$	0.150	(~1)
2.8	$0.045 \pm 0.031$	0.137	(~1)
78.4	$0.035 \pm 0.032$	0.250	(~1)
<b><math>U_0</math> Stokes parameters</b>			
39.2§	$0.016 \pm 0.034$	0.569	(~1)
9.8§	$0.017 \pm 0.033$	0.554	(~1)

\*As furnished by statistical program, with pre-assigned period or periods; equals twice the product of probabilities for sine and cosine terms.

†Simple probability multiplied by  $(192/2)$ , the number of statistically independent frequencies or periods obtainable with 192 data points. Meaningful for small probabilities ( $\ll 1$ ).

‡Based on 3-parameter fit: slope plus 39.2 d sine, cosine.

§From 5-parameter fit: slope plus 39.2 d and 9.8 d sines, cosines.

||From 5-parameter fit: slope plus 39.2 d and harmonic or sub-harmonic sines, cosines.

## Possible 39-d polarisation period in Cyg X-1

KEMP<sup>1,2</sup> has reported that the power spectra of the  $Q$  and  $U$  Stokes polarisation parameters, for the  $U$  band radiation from Cyg X-1, show a  $\sim 3\sigma$  peak at  $39.26 \pm 0.10$  d. The peak was found to represent a modulation in which  $Q$  and  $U$  vary, in phase, at 0.27% peak-to-peak total polarisation. While this was reported to be the strongest  $U$  band periodicity in the frequency range considered, it was not found in the  $B$  and  $V$  bands (ref. 3 and J. Kemp, personal communication). Further

observations are necessary to set limits on this modulation for the various radiation bands. This letter suggests that such variable polarisation component may arise from those optical photons which, after being radiated by the disk, are Thomson scattered into our line of sight by the flared material at the outer parts of the disk and in the incoming flow<sup>4</sup>. Details will be published elsewhere<sup>5</sup>. Here we note that, with a favourable ratio of electron scattering opacity to absorption opacity and with a scattering depth  $\tau_{es} \sim 1$ , such scattering can polarise the photons to a high degree ( $\sim 20\%$ ); we also note, that the in-phase variability of  $Q$  and  $U$  can come about if the azimuthal structure of the scattering region does not have a 39.2-d variation (for example, if the disk is always axially symmetrical), while the  $r, z$  structure does have sufficient variability to change the amount of scattered photons relative to the total flux and hence to produce a variation in total polarisation.

Matter from the primary which flows through L1 to join the outer disk assumes, on a Keplerian time scale, such constant azimuthal structure, which we may call 'the scattering rim'.  $r, z$ -Structural variability can then come about by a modulation in L1 overflow. The latter can be induced by tidal interaction with an eccentric, 39-d periodic motion of a third, light, star around the Cyg X-1/HDE 226868 system, in a way similar to that suggested previously for the Her X-1/HZ Her system<sup>6</sup>.

The L1 point for the binary system is located  $\sim 10\text{--}16 \times 10^{11}$  cm from the compact object, for the accepted range of binary masses. If the rim is located at a distance of  $r_{11} \times 10^{11}$  cm and, for simplicity, extends over  $0.2 \times 10^{11}$  ( $\alpha/0.2$ )  $r_{11}$  cm in the  $r$  and  $\pm z$  directions, then its transversal optical scattering depth is  $\tau_{es} \sim 3(\alpha/0.2)^{-1} \psi r_{11}^{-2} \dot{m}_{-9}$ , where  $\dot{m}_{-9}$  is the average accretion rate, in units of  $10^{-9} M_{\odot} \text{yr}^{-1}$ , and  $\psi$  is the mass of the 'rim' in units of the total mass accreted during a 39-d period. In steady state, the inward drift time of the 'rim' is of order  $\sim 39$  d and we expect  $\psi \lesssim 1$  for a large flow modulation. Due to the non-hydrostatic nature of the rim<sup>7</sup> and its heating<sup>8</sup>, we expect  $\alpha$  to be larger than the angular size calculated for hydrostatic disks. The condition  $\tau_{es} \sim 1$  thus seems to be compatible with current understanding of disk and mass flow (when  $\tau_{es} > 1$ , (but not  $\gg 1$ ) polarisation by backscattering could still occur).

For U band photons,  $\kappa_{es}$  dominates at densities

$$\rho < 2 \times 10^{-10} T_4^{1/2} \text{ g/cc} = \rho_c \quad (1)$$

where  $T_4$  is the temperature in  $10^4$  K. On rewriting equation (1) as  $T_4 \geq 0.5 r_{11}^{-2} (\alpha/0.2)^{-2} \tau_{es}^2$ , we note that this condition is also likely to be satisfied, since rim cooling drops sharply when  $T_4 < 1$  due to the decreasing ionisation, and since the rim is being constantly heated by the incident X-ray flux and the jet-disk shock.

Due to uncertainties in efficiency and total luminosity of Cyg X-1 we can estimate only roughly the  $U$  luminosity,  $L_{u,d}$ , in the direction of the rim. To produce a 0.27% total polarisation with 20% scattering polarisation we must have  $L_{u,d} \sim 0.1(\alpha/0.2)^{-1} L_{u,p} \sim 0.2(\alpha/0.2)^{-1} L_{x,d}$ , where  $L_{u,p}$  is the primary luminosity and  $L_{x,d}$  is the 2–6 keV X-ray luminosity<sup>9</sup>. Since most of the optical luminosity is due to X-ray heating<sup>10</sup>, we do expect a suppression of  $B$  and  $V$  rim scattered flux, hence of 39-d  $B$  and  $V$  polarisation modulation, relative to the  $U$  band. This is because such optical surface emission has a strong limb brightening effect<sup>11</sup>, especially at glancing angles, and may develop a marked inverted Balmer jump (higher intensity for wavelengths below the Balmer edge). Some support for this property of heated surface emission is furnished by indirect observations of the continuum emission from the disk in SMCX-1 (ref. 12).

The existence of a modulated rim structure can be tested by further observations: The hard X-ray ( $> 10$  keV) intensity and polarisation should be modulated at 39 d, with respective peak-to-peak amplitudes of 70% and 15%, where  $a$  is the albedo. The average polarisation for  $a \sim 0.2$  and  $a \sim 0.5$  is comparable to the predicted<sup>5,13</sup> and observed<sup>14</sup> constant

polarisation of hard X rays. The corresponding amplitudes for soft X rays should be smaller due to their increased absorption. Furthermore, a 0.015 mag peak-to-peak 39 d modulation in  $U$  intensity is expected, with a smaller amplitude for the  $B$  and  $V$  radiations. All of these modulations should occur in phase, and, to the extent that the rim structure changes during a high-low transition in Cyg X-1<sup>15</sup>, their amplitudes should change too.

The existence of a third star can be further tested by observations of additional periodicities in intensities and polarisations. Aside from smaller amplitude binary or half binary periods, periods associated with the variable tidal forces are expected. The latter form a large set of sums of integral multiples of  $\frac{1}{2}(\omega_b - \omega_3)$  and the 39 d frequency, where  $\omega_b$  is the binary frequency and the  $\omega_3$ 's are instantaneous frequencies of the third stellar motion. Such tidal modulations will only show up as flow modulations if the time responses at L1 and during the free-fall, circularisation and inward rim diffusion are sufficiently small. The proximity of the observed 39.2-d period to an integral multiple of half a binary period ( $14 \times 2.8$  d) may be due to the eccentricity of the binary orbit or may be related to the forbidden binary phase for X-ray turn-ons in Her X-1 (see ref. 6). We also expect longer periods, associated with modulations in binary eccentricity and precession of its nodes.

Similar polarisation measurements for the Her X-1/HZ Her system may distinguish between models suggested for its 35-d period. For example, if  $U$  and  $Q$  are found there too to vary in phase, this may rule out a precessing disk model.

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1. Kemp, J. C. *IAU Circ.* no. 3060 (1977).
2. Kemp, J. C., Herman, L. C., Rudy, R. J. & Barbour, M. S. *Nature* 270, 227–228 (1977).
3. Kemp, J. C., Southwick, R. G. & Rudy, R. J. *Astrophys. J.* 210, 239–249 (1976).
4. Rees, M. J. *Mon. not R. astr. Soc.* 171, 457–465 (1975).
5. Milgrom, M. & Shaham, J. *Astr. Astrophys.* (to be submitted).
6. Mazeh, T. & Shaham, J. *Astrophys. J. Lett.* 213, L17–L20 (1977).
7. Lubow, S. H. & Shu, F. H. *Astrophys. J. Lett.* 207, L53–L55 (1976).
8. Hatchett, S., Buff, J. & McCray, R. *Astrophys. J.* 206, 847–860 (1976).
9. Heise, J. *et al. Nature* 256, 107–108 (1975).
10. Cunningham, C. *Astrophys. J.* 208, 534–549 (1976).
11. Milgrom, M. *Astr. Astrophys.* 50, 273–277 (1976).
12. van Paradijs, J. & Zuiderwijk, E. *Univ. Amsterdam preprint* (1977).
13. Lightman, A. P. & Shapiro, S. L. *Astrophys. J.* 203, 701–703 (1976).
14. Novick, R. *6th Texas Symp. Relativistic Astrophys.* (Boston, 1976).
15. Ichimaru, S. *Astrophys. J.* (in the press).

## The 39-d period in Cyg X-1

KEMP *et al.*<sup>1,2</sup> recently claimed to have discovered a 39-d period in the linear polarisation of the U band light for HDE 226868, the optical counterpart of Cygnus X-1. Within the errors their period is exactly seven times the 5.60-d orbital period of this binary system. Based on this result Milgrom and Shaham<sup>3</sup> produced a model for Cyg X-1 involving a three-body system in which the third component is in a 39-d orbit about the 5.6-d X-ray binary system. This note draws attention to the absence of a 39-d period in other data on this star.

Walker and Rolland Quintanilla<sup>4</sup> have recently completed an analysis of 349 nights of B band data obtained in 1972–76.



Two methods were used to search these data for possible periods. The first was based on the method of Lafler and Kinman<sup>5</sup> and the second on the Fourier technique developed by Grey and Desikachary<sup>6</sup>. Neither method shows any evidence for a period of 39 d or at one half or double that period. A realistic upper limit can be set to any modulation of the blue light of this star at any of these periods of 0.001 mag., that is, 0.1%. This has to be compared with the modulation at 2.8 d (half the orbital period) of over 2%.

A literature search for periods in 215 radial velocities for this star<sup>7-13</sup> allows us to put an upper limit on any 39-d period of  $<10 \text{ km s}^{-1}$  compared with over  $60 \text{ km s}^{-1}$  for the variations with the 5.60-d orbital period.

We have also searched the X-ray flux measurements for periods using the Grey and Desikachary<sup>6</sup> method. The data are from the All Sky Monitor (2–6 keV) and the Sky Survey Instrument (2–18 keV) on the Ariel V satellite. Combined data from both experiments give almost complete coverage at one satellite orbit interval ( $\sim 100 \text{ min}$ ) over the 590 d before March 1977 including both high and low states of X-ray activity. The high state data are characterised by massive (approximately fourfold) modulation of an apparently random character and an upper limit of  $\sim 10\%$  is the best that can be placed on any 39-d modulation. But, the much more extensive low state data, where the overall modulation is much smaller, give an upper limit of  $\sim 3\%$  to any 39-d modulation. In both cases the power at  $\sim 39 \text{ d}$  is significantly less than that at nearby frequencies and in neither case does the power spectrum suggest any significant 39-d period.

We believe that the reality of this period and the subsequent model must be in doubt. This is because of the marginal nature of the evidence for a 39-d period in the U-band polarimetry and the upper limits to modulation with this period, or its nearest harmonics, in the B band, radial velocity and low state X-ray data.

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1. Kemp, J. C. *I.A.U. Circ.* No. 3060 (1977).
2. Kemp, J. C., Herman, L. C., Rudy, R. J. & Barbour, M. S. *Nature* **270**, 227–228 (1977).
3. Milgrom, M. & Shaham, J. *Nature* **270**, 228–229 (1977).
4. Walker, E. N. & Rolland Quintanilla, A. *Mon. Not. R. star. Soc.* (in the press).
5. Lafler, J. & Kinman, T. D. *Astrophys. J. Suppl.* **11**, 216–222 (1965).
6. Grey, D. F. & Desikachary, K. *Astrophys. J.* **181**, 523–530 (1973).
7. Bolton, C. T. *Nature* **235**, 271–273 (1972).
8. Bolton, C. T. *Nature phys. Sci.* **240**, 124–127 (1972).
9. Smith, H. E., Margon, B. & Conti, P. S. *Astrophys. J. Lett.* **179**, L125–L128 (1973).
10. Webster, B. L. & Murdin, P. G. *Nature* **235**, 37–38 (1972).
11. Hutchings, J. B., Crampton, D., Glaspey, J. & Walker, G. A. H. *Astrophys. J.* **182**, 549–557 (1973).
12. Brucato, R. J. & Zappala, R. R. *Astrophys. J. Lett.* **189**, L71–L74 (1974).
13. Mason, K. O., Hawkins, F. J., Sanford, P. W., Murdin, P. & Savage, A. *Astrophys. J. Lett.* **192**, L65–L69 (1974).
14. Bolton, C. T. *Astrophys. J.* **200**, 269–277 (1975).
15. Abt, H. A., Hintzen, P. & Levy, S. G. *Astrophys. J.* **213**, 815–817 (1977).

## Mercury in surface waters of seas around the United Kingdom

A SURVEY of the concentration of mercury in the surface waters of the seas around the UK, has been made as part of the Ministry of Agriculture, Fisheries and Food's overall programme of investigation into the distribution of heavy metals in the marine environment. Using a cold vapour–

gold trap method on board a research vessel, thus obviating the necessity to store the samples with consequent problems of loss and/or contamination, both 'reactive' and 'total' mercury concentrations have been measured. The results reported here show clearly the major sources of emission. The possible reasons for differences between these results and those of some other investigators are also discussed.

Preston *et al.*<sup>1</sup> have described a pilot survey carried out to measure the concentrations of selected heavy metals in seawater, suspended matter and biological indicators from British coastal waters. Since then analytical methods have been refined and more surveys carried out. Recently, because of the significance of possible mercury pollution and its effect on man, surveys have been carried out to measure the mercury levels in UK coastal waters. Because of the problems of loss of mercury and contamination of samples with mercury<sup>2,3</sup>, we have analysed such samples immediately after collection. The technique described here is designed for use on board a research vessel.

The sample, collected either by plastic bucket or Niskin bottle and not filtered, is immediately transferred to a 2.5 l glass bottle, previously checked for contamination, and acidified by adding 10 ml of concentrated sulphuric acid per l of sample. A 100 ml aliquot of this is taken for analysis. The mercury is swept from solution, after reduction with stannous chloride, by bubbling nitrogen through the sample and collecting the mercury on gold wire chips. The mercury is then determined by heating the gold to high temperature, and sweeping the mercury through a Coleman MAS 50 mercury analyser. The resultant absorption peak is recorded on a strip chart recorder set at about a  $\times 30$  expansion of the MAS 50 output. The peak height is measured and compared with a standard calibration line produced either by internal standardisation, or addition of mercury standards to a previously reduced sample. The practical detection limit is about  $0.5 \text{ ng l}^{-1}$  and the coefficient of variation at  $1 \text{ ng l}^{-1}$  level is about  $\pm 10\%$ . The analysis takes 10 min from the time the sample arrives on deck.

The results of the analysis of samples taken on two cruises during 1976 are given in Fig. 1. The cruises were carried out between 2 and 16 January, a period of extremely stormy weather, and between 25 May and 14 June, a period of prolonged calm weather, when the suspended load was abnormally low. The areas have been chosen arbitrarily, and the individual high spots have not been included in the calculation of the mean value for each area. Figure 2 shows contours derived from the data summarised in Fig. 1 and the positions of the sampling stations. The main areas of mercury contamination are apparently in the Thames Estuary, Liverpool Bay, the area off the Humber Estuary and, to a lesser extent, the Bristol Channel and the mouths of the Rhine and Elbe rivers. The data from areas A, B, C, D, H and L do not show any clear out contouring.

The exact forms in which mercury exists in seawater are in doubt, but it is likely that as well as being present as an anionic chloride complex, it will also be complexed by organic material both in solution and in suspended matter. How filtering affects the mercury content of the sample is uncertain, so an environmentally artificial, analytical delineation between 'reactive' and 'total' mercury has been conveniently adopted. The 'reactive' mercury is defined as that which is available for reduction by stannous chloride from samples acidified to about pH 1. The 'total' mercury is that made available to reduction by stannous chloride, following oxidation of the sample with ammonium persulphate. Chlorine, produced during the oxidation, interferes with the measurement, and is removed by a ferrous sulphate scrubber after the reduction step. The mean values obtained for total mercury levels are given in Table 1. The values for the areas H, I and J are not available due to difficulties experienced with the oxidation stage during the early part of each

cruise. In every case an increase in the mercury measured after oxidation was observed.

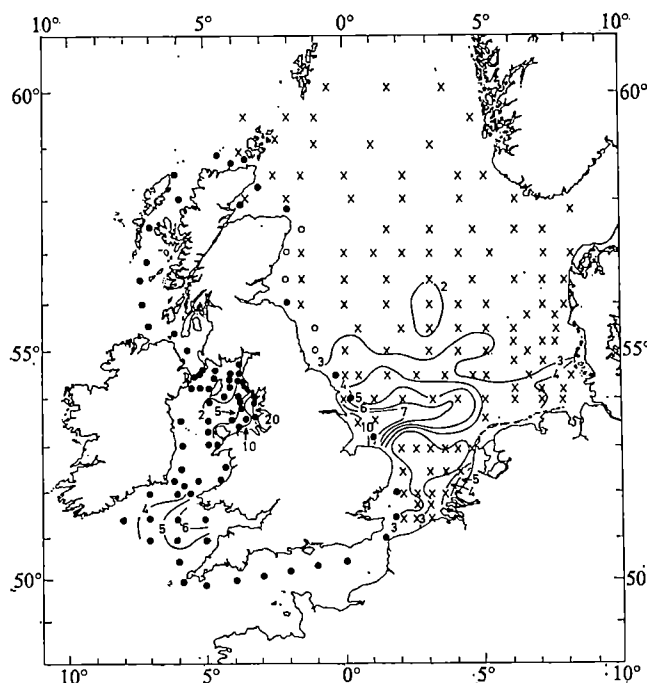
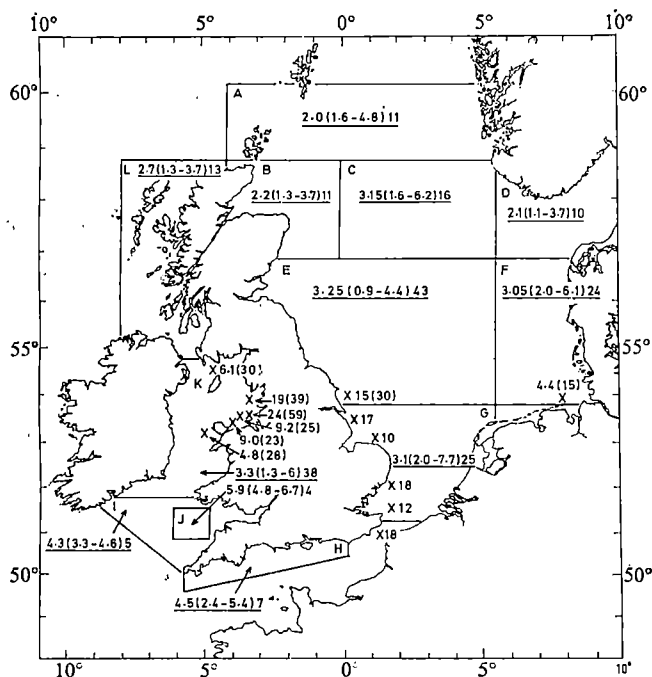
Samples collected in the open ocean areas, acidified and analysed immediately, stored for 1 month in glass and then reanalysed, gave similar results for both the 'reactive' and the 'total' mercury. One sample, however, collected during 1975, in an area of high suspended load and high mercury contamination gave results of  $8 \text{ ng l}^{-1}$  and  $73 \text{ ng l}^{-1}$  for 'reactive' and 'total' mercury respectively, and 1 month later  $58 \text{ ng l}^{-1}$  and  $75 \text{ ng l}^{-1}$  respectively. Samples which have been treated by boiling with acid alone have also shown an increase in measured mercury, to a level which is not as high as that obtained by oxidation.

In samples collected at offshore stations no attempt was made to distinguish between mercury present in solution and that associated with any particulate material present. But, one sample (collected during 1975, in an estuary where pollution does occur and containing a very high suspended load) was filtered through a  $0.22\text{-}\mu\text{m}$  filter membrane; and on analysis  $17 \text{ ng l}^{-1}$  were found in the filtrate and  $950 \text{ ng}$  were found in the particulate associated with that 1-l sample.

Conclusions from these data are that the mercury present in seawater samples is complexed to varying degrees and that a measurement of the total mercury present requires a vigorous oxidation step to make the mercury available for reduction by stannous chloride in cold vapour measurement techniques.

Samples collected in open ocean areas acidified and stored in glass containers are stable for at least 1 month. In samples collected in areas of high suspended load, however, a change in the ratio of 'reactive' to 'total' mercury may occur on storage in acid conditions, although the total mercury is unchanged. In addition a large proportion of mercury present in samples of high suspended load is associated with the particulate matter. The fact that apparently higher levels were found in the Irish Sea than the North Sea may be an artefact of the sampling conditions and proximity to the point of discharge. Those in the western and southern

**Fig. 1** Concentration of reactive mercury ( $\text{ng l}^{-1}$ ) from two surveys in 1976. Values given are mean concentration (range of values) and no. of samples analysed. Positions marked, for example,  $\times 15$  are position of sampling and concentration measured. Where total mercury is also available positions are marked, for example,  $\times 15$  (30).



**Fig. 2** Reactive mercury ( $\text{ng l}^{-1}$ ). Contoured concentration from two surveys (see Fig. 1).  $\bullet$ , positions from January 1976 cruise;  $\times$ , from May-June 1976 cruise.

areas around the UK and the isolated 'high spot' points off the Thames Estuary were collected during a period of extreme storms and consequently high suspended load. The Liverpool Bay samples were collected closer to any point of discharge than, for example, those samples collected off the mouths of the Elbe and Rhine. It may also be significant that in the areas where the levels of mercury are high, sewage sludge is dumped.

The mean and range of values for 'total' mercury concentrations, excluding those individual high spots in the vicinity of discharge points,  $7.9$  ( $3.4\text{--}22$ )  $\text{ng l}^{-1}$ , compare favourably with those obtained by Fitzgerald and Lyons<sup>5</sup> for the North-West Atlantic,  $8$  ( $\pm 3$ )  $\text{ng l}^{-1}$ . Leatherland *et al.*<sup>6</sup>;  $13$  ( $<3\text{--}20$ )  $\text{ng l}^{-1}$ , for the North-East Atlantic; and Burton and Leatherland<sup>7</sup>,  $11\text{--}28$   $\text{ng l}^{-1}$ , for the English Channel. Fitzgerald and Lyons<sup>5</sup> use a method very similar to that described here, but the others use one based on a final colorimetric measurement after concentration by ion exchange.

In contrast, the concentrations measured by Chester *et al.*<sup>8</sup>,  $47$  ( $<0.5\text{--}127$ )  $\text{ng l}^{-1}$  for the world's oceans and by Fitzgerald *et al.*<sup>9</sup>  $140$  ( $\pm 70$ )  $\text{ng l}^{-1}$  for the North-West Atlantic, have much higher means and are much more widely spread. We have not found such high values nor so widely ranged ones, even in areas of obvious contamination.

As with all measurements of heavy metals in seawater samples, great care must be taken to avoid contamination during collection, and loss and/or contamination during storage. Polythene bottles are notoriously contaminated with mercury. It is possible that those investigators who use cold vapour detectors may well be measuring parameters unrelated to the mercury content of the sample. The addition of nitric acid to polythene bottles may cause the release of some organic constituent of the plastic which will absorb at the wavelength used to determine the mercury. Chlorine, produced by oxidising the sample, will certainly interfere with cold vapour detection, and this source of error will have been missed by those investigators who make blank determinations using distilled water in place of 'mercury free' seawater. Despite great efforts to clean it, glassware

**Table 1** Concentrations of total mercury levels where available and ratio of reactive to total mercury for individual areas

Area	Mean total Hg (ng l <sup>-1</sup> ) (range)	Reactive Total
A	6.1 (3.4-9.5)	0.33
B	6.4 (3.9-11)	0.34
C	7.3 (4.3-12)	0.43
D	6.8 (4.8-9.1)	0.31
E	6.6 (3.5-11)	0.50
F	7.5 (5.2-11)	0.41
G	7.9 (5.6-11)	0.39
K	13 (6.0-22)	0.25
L	9.6 (4.8-20)	0.28

used in the oxidation step, will over a very long period, continue to release mercury into the samples, therefore we use quartz vessels during the oxidation step.

Matsunaga *et al.*<sup>10</sup> measured the 'reactive' mercury concentrations in seawater and reported values of 5 (3.6-5.6) ng l<sup>-1</sup> which are in good agreement with the values obtained in this survey, 3.3 (0.9-7.7) ng l<sup>-1</sup>. They did not, however, oxidise their samples and had they done so higher values would have resulted.

The ratios of 'reactive' to 'total' mercury present are similar to those obtained by Fitzgerald and Lyons<sup>1</sup> although the concentrations in this survey were of an order of magnitude lower.

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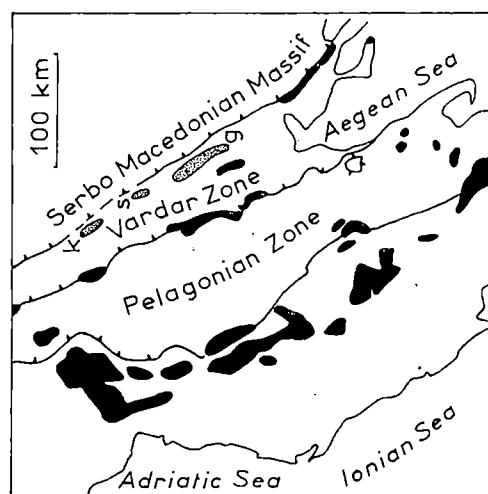
1. Preston, A., Jefferies, D. F., Dutton, J. W. R., Harvey, B. R. & Steele, A. K. *Environ. Pollut.* 3, 69-82 (1972).
2. Fitzgerald, W. F., Lyons, W. B. & Hunt, C. D. *Analyt. Chem.* 46, 1882-1885 (1974).
3. Robertson, D. E. *Pacific North-west Laboratory, Annual Report 1974*. BNWL 1950, part 2, UC-48, 141-142 (1974).
4. Fitzgerald, W. F. & Lyons, W. B. *Nature* 242, 452-453 (1973).
5. Fitzgerald, W. F. & Lyons, W. B. *Limnol. Oceanogr.* 20, 468-471 (1975).
6. Leatherland, T. M., Burton, J. D., McCartney, M. J. & Culkin, F. *Nature* 232, 112 (1971).
7. Burton, J. D. & Leatherland, T. M. *Nature* 231, 440-441 (1971).
8. Chester, R., Gardner, D., Riley, J. P. & Stoner, J. *Mar. Pollut. Bull.* 4, 28-29 (1973).
9. Fitzgerald, R. A., Gordon, D. C. Jr. & Cranston, R. E. *Deep-Sea Res.* 21, 139-144 (1974).
10. Matsunaga, K., Nishimura & Konishi, S. *Nature* 258, 224 (1975).

## Mafic and ultramafic rocks associated with granites in the Vardar zone

SEVERAL ophiolitic alignments have been classically recognised in the Dinarides and the Hellenides (Fig. 1). The Eastern alignment, located near the Serbo-Macedonian massif in the Vardar zone, shows curious associations of mafic and ultramafic rocks with granites. Here a brief description of the Guevgueli magmatic complex which belongs to the Eastern alignment helps illustrate this peculiarity: petrographic and major element data seem to prove that these associations occur on the border of opening inter-arc basins.

The Guevgueli complex, in Greek Macedonia, is a large thrust slice within Jurassic sedimentary and volcanic formations<sup>1</sup>. A fault separates this complex into two petrographically distinct units (Fig. 2)<sup>2</sup>. (1) The Western unit is built up of plutonic rocks—gabbroic cumulates, high Fe and Ti diorites (FeO\* 18%; TiO<sub>2</sub> 5%) (FeO\*: total iron as FeO), quartz-diorites and rare trondhjemites—separated from lavas by a continuous layer of hypabyssal formations. All these rocks have a very low K<sub>2</sub>O content and present a tholeiitic differentiation trend<sup>3</sup> with a marked enrichment in FeO\* and TiO<sub>2</sub>.

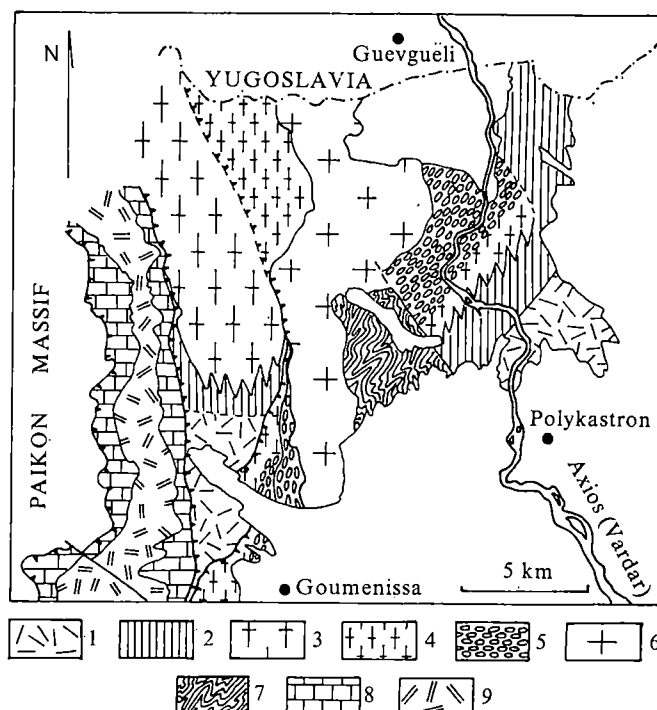
(2) The Eastern unit is more varied: it is made up of lavas, hypabyssal rocks forming a sheeted



**Fig. 1** Structural map of the central part of the Dinarides and the Hellenides. Black areas, ophiolites; stippled area associations of mafic and ultramafic rocks with granites (k, Karadagh; s, Stip; g, Guevgueli).

complex, and gabbroic cumulates cut by abundant magmatic breccias in which dolerites, gabbros, diorites, tonalites, trondhjemites and granites are closely associated. The sheeted complex has a calc-alkaline affinity<sup>3</sup>, whereas the magmatic breccias present a tholeiitic differentiation trend with enrichment in FeO\* and TiO<sub>2</sub>. These rocks are in tectonic contact with biotite-orthoclase-sillimanite-cordierite migmatites. A granite massif (the Fanos Granite) is intrusive into the migmatites, the gabbroic cumulates and the magmatic breccias; the biotites of this granite, dated by K-Ar and Rb-Sr methods, are 150 Myr old (Upper Jurassic)<sup>4</sup>.

The Eastern unit seems to result from the juxtaposition of two groups of magmatic rocks. The first one is made up



**Fig. 2** Geological map of the Guevgueli complex. 1, lavas; 2, hypabyssal rocks; 3, gabbroic formations (Western unit); 4, gabbroic formations (Eastern unit); 5, magmatic breccias; 6, Fanos Granite; 7, migmatites; 8, Mesozoic limestones; 9, volcano-sedimentary formations rich in rhyolites.

of basic, intermediate and acid formations with low  $K_2O/Na_2O$  ratios; the second one consists of granitic rocks with  $K_2O/Na_2O > 1$ . This juxtaposition can be seen in the magmatic breccias, in the sheeted complex which includes microgranitic dykes, and in lavas in which rhyolites are present. These observations lead to the conclusion that the low and high  $K_2O$  formations are contemporaneous.

Ultramafic rocks are scarce in the Greek part of the Guevgueli complex, but they outcrop on the other side of the border in Yugoslavia<sup>5</sup>. Moreover, the Yugoslavian magmatic complexes of Karadagh, near Skopje, and Stip<sup>6,7</sup>,

the  $TiO_2$  content and the  $FeO^*/MgO$  ratio, for low values of  $FeO^*/MgO$ , in abyssal tholeiites; this positive correlation does not appear, or is very weak, in low  $K_2O$  island-arc tholeiites (Fig. 3). The variations of the  $TiO_2$  contents with the  $FeO^*/MgO$  ratio in hypabyssal rocks of the Western unit and magmatic breccias of the Eastern unit of the Guevgueli complex, in dolerites and lavas of the Yugoslavian Karadagh complex, and in abyssal tholeiites are comparable (Fig. 3).

The magmatic complexes of the Eastern ophiolitic alignment in the Dinarides and the Hellenides, therefore, show a

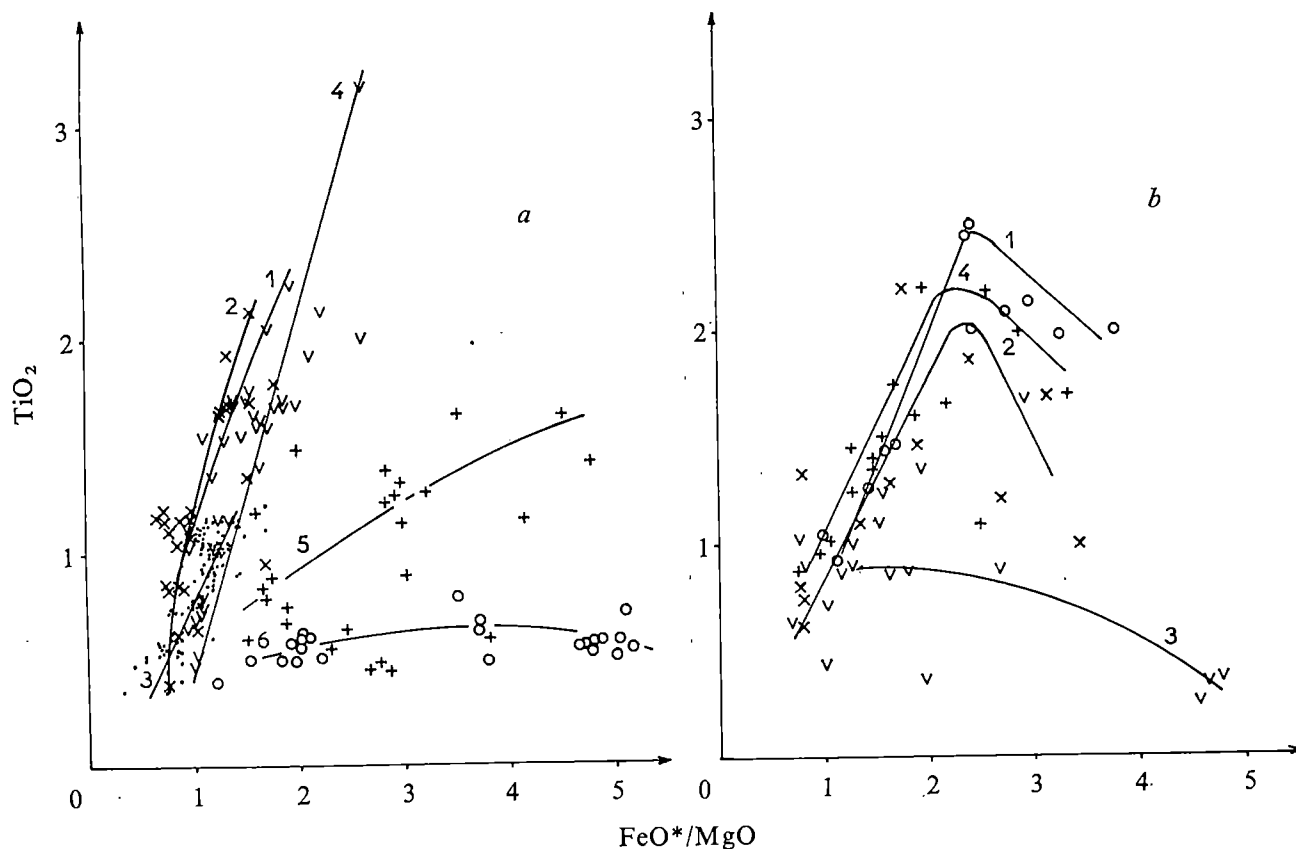


Fig. 3  $TiO_2$ ,  $FeO^*/MgO$  diagrams *a*, of recent volcanic rocks; 1, abyssal tholeiites<sup>8</sup>; 2 ( $\times$ ), basalts from the Lau Basin, an interarc basin between the Tonga and the Lau Ridges<sup>11</sup>; 3 ( $\bullet$ ), basalts from Leg 37 of the Deep Sea Drilling Project (abyssal tholeiites with very low  $TiO_2$  content)<sup>12</sup>; 4 ( $\nabla$ ), basalts from Iceland<sup>13</sup>; 5 ( $+$ ), low  $K_2O$  tholeiites from Hachijo-Jima, Izu-Bonin Arc<sup>14</sup>; 6 ( $\circ$ ): low  $K_2O$  tholeiites from Tonga<sup>15</sup>. *b*, Of magmatic formations of the Eastern ophiolitic alignment in the Dinarides and the Hellenides: 1 ( $\circ$ ), hypabyssal rocks, Western unit, Guevgueli complex; 2 ( $\times$ ), magmatic breccias, Eastern unit, Guevgueli complex; 3 ( $\nabla$ ), hypabyssal rocks, Eastern unit, Guevgueli complex; 4 ( $+$ ), dolerites and lavas, Yugoslavian Karadagh Massif<sup>6</sup>.

located near the Serbo-Macedonian massif, also show close associations of mafic and granitic rocks and include important masses of ultramafic formations (dunites with chromite, lherzolites, harzburgites, pyroxenites).

Thus, all these magmatic complexes exhibit the juxtaposition of distinct associations. The abundance of granites does not seem compatible with an oceanic setting for these rocks. The presence of formations with calc-alkaline affinity (sheeted complex of the Eastern unit) is classically considered to be characteristic of island arcs and active continental margins<sup>8</sup>. Two kinds of magmatic rocks can be compared to the tholeiitic series with low  $K_2O$  content of the Guevgueli complex: low  $K_2O$  island-arc tholeiites and abyssal tholeiites. The similarity between these formations has caused many discussions on the origin of some ophiolitic complexes. It seems that the  $TiO_2$  content can resolve this problem. The low  $K_2O$  tholeiites of island-arcs usually have slightly lower  $TiO_2$  contents than common abyssal tholeiites, but there are many important exceptions<sup>9</sup>. On the other hand, a marked positive correlation can be observed between

close relation in space and time between abyssal tholeiites, island-arc magmatism and granites. Such associations are found on the border of inter-arc basins; so, it seems that the magmatic rocks of the Guevgueli and Karadagh complexes were formed during the creation of inter-arc basins in the Vardar zone. The ultramafic and mafic formations with tholeiitic affinity are relics of the crust and the mantle of inter-arc basins; the calc-alkaline and granitic rocks are the results of the magmatic activity on the borders of these opening basins. This interpretation, if true, is important for our understanding of the structure<sup>10</sup> of the Dinarides and the Hellenides.

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1. Mercier, J. L. *Annls géol. Pays Hel.* 20, 792 (1968).
2. Bebie, J. & Mercier, J. L. *Bull. Soc. Géol. Fr.* (in the press).



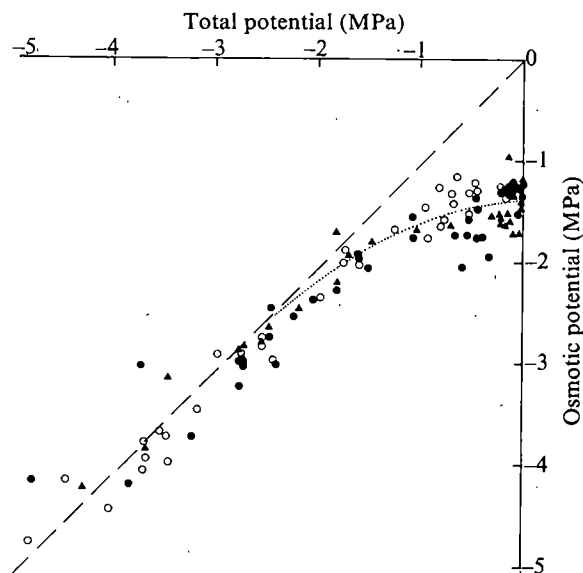
3. Bebie, J., Ohnenstetter, D., Ohnenstetter, M., Paupy, A. & Rocci, G. *Pétrologie* 1, 157-168 (1975).
4. Borsi, S., Ferrara, G., Mercier, J. L. & Tongiori, E. *Revue Géogr. phys. Géol. Dyn.* 8, 279-287 (1966).
5. Effendiantz, L. thesis, Univ. Besançon (1971).
6. Rollet, M. thesis, Univ. Besançon (1969).
7. Bulle, J. & Rollet, M. *Bull. Soc. Géol. Fr.* 12, 1048-1059 (1970).
8. Miyashiro, A. *Earth planet. Sci. Lett.* 19, 218-224 (1973).
9. Church, W. R. & Colish, R. A. *Earth planet. Sci. Lett.* 31, 8-14 (1976).
10. Mercier, J. L., Vergely, P. & Bebie, J. C. R. *Séanc. Soc. Géol. Fr.* 108-112 (1975).
11. Hawkins, J. W. Jr. *Earth planet. Sci. Lett.* 28, 283-297 (1976).
12. Blanchard, D. P. *et al. J. geophys. Res.* 81, 4231-4246 (1976).
13. Sigvaldson, G. E. *J. Petrol.* 15, 497-524 (1974).
14. Isshiki, N. *Tokyo Univ. Fac. Sci. J.* 15, 91-134 (1963).
15. Ewart, A., Bryan, W. B. & Gill, J. B. *J. Petrol.* 14, 429-465 (1973).

## Differences in osmoregulation between wheat genotypes

SEVERAL recent experiments provide evidence to support the existence of osmoregulation in plants as a means of counteracting water stresses induced by changes in the soil or evaporative environment<sup>1-3</sup>. Viewed in terms of energy potentials, decreases in the water potential,  $\Psi$ , induced by changes in the environment, are immediately offset by decreases in the osmotic potential,  $\pi$ , through an increase in solute content. The turgor potential,  $P$ , is thereby maintained since  $\Psi = \pi + P + \tau$  (assuming that the matric potential,  $\tau$ , is small or constant). The importance of this response is that expansion growth, which is affected directly by the turgor potential, is maintained over a range of values of  $\Psi$ <sup>2</sup>. Despite this obvious significance, little is known of the extent of this phenomenon in crop plants in general and in particular of differences between genotypes in the same or closely related species. Here we present evidence indicating substantial differences between several genotypes of wheat; in some osmoregulation was marked, whereas in others it was virtually non-existent.

To examine a wide spectrum of the possible variation, the following genotypes were selected: *Triticum aestivum* spp. *vulgare* (AUS 2067), *T. aestivum* spp. *spelta* (AUS 3843), *T. aestivum* spp. *vulgare* (AUS 3850), *T. durum* (AUS 2816), *T. dicoccum* (AUS 3582) and *T. boeoticum* spp. *aegelopoides* (AUS 90352). After vernalising the plants were grown in pots in a glasshouse with temperatures 20-25 °C during the day and 10-15 °C during the night. Water stresses were produced by withholding water at two stages of development: about 14 d before and 14 d after anthesis. Plants were placed in a controlled environment chamber adjusted to give a flux density of total short wave radiation of 140 W m<sup>-2</sup> at the average plane of the flag leaves and temperatures of 23 °C during the light period

Fig. 1 Relationship of leaf water potential to osmotic potential. The broken line is the line of equality.  $\blacktriangle$ , *T. durum* (AUS 2816);  $\circ$ , *T. aestivum* spp. *vulgare* (AUS 2067);  $\bullet$ , *T. boeoticum* (AUS 90352).



(duration 14 h) and 15 °C during the dark period. Total potential  $\Psi$  was measured on samples of the flag leaves using thermocouple psychrometers and  $\pi$  was measured in the same way after the samples had been frozen in liquid nitrogen<sup>4</sup>. Relative water contents were measured using the technique of Slatyer and Barrs<sup>5</sup>.

When  $\Psi$  falls in response to a reduction in soil water supply, it is usual for  $\pi$  to fall at a slower rate, such that there is a linear decline in the turgor potential until  $\Psi = \pi$ . This pattern has been observed for a range of different plant species, including wheat<sup>6-8</sup>. The response of the genotypes *T. aestivum* spp. *vulgare* (AUS 2067), *T. durum* (AUS 2816), *T. boeoticum* spp. *aegelopoides* (AUS 90352) fit this pattern (Fig. 1). The osmotic potential fell from a value of about -1.5 MPa at full turgor to a value of about -2.5 MPa at zero turgor. This was very similar to the pattern observed in a previous experiment<sup>7</sup>. When osmoregulation occurs we expect  $\pi$  to decrease with  $\Psi$  so as to maintain  $P$ . This was in fact observed for the genotypes *T. aestivum* spp. *vulgare* (AUS 3850), *T. dicoccum* (AUS 3582), and *T. aestivum* spp. *spelta* (AUS 3843) (Fig. 2). Here, over the range of  $\Psi$  for which the leaf had positive turgor,  $\pi$  declined, from an initial value of about -1.5 MPa, at approximately the same rate as  $\Psi$ , thus maintaining  $P$ . At the end of this period of adjustment, when  $\Psi$  had reached about -1.5 MPa and  $\pi$  about -3.0 to -3.5 MPa,  $\pi$  equilibrated rapidly with  $\Psi$ . This phenomenon was observed at both stages of development.

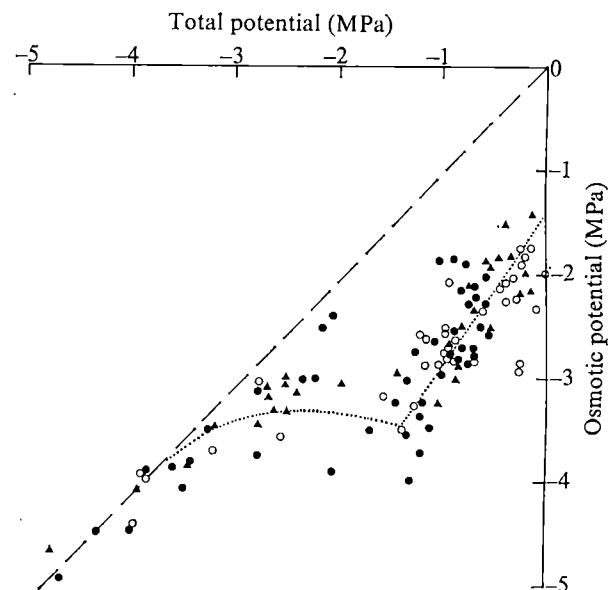


Fig. 2 Relationship of leaf water potential to osmotic potential. The broken line is the line of equality.  $\blacktriangle$ , *T. aestivum* spp. *vulgare* (AUS 3850);  $\circ$ , *T. dicoccum* (AUS 3582);  $\bullet$ , *T. aestivum* spp. *spelta* (AUS 3843).

If the solute content of the leaves did not vary with the relative water content,  $\zeta$ , then  $\pi$  should be altered by gains or losses of water according to the equation<sup>9</sup>

$$\pi = (\pi_t \zeta_t) / \zeta \quad (1)$$

or if bound water is present,  $(\pi + \tau) = (\pi_t + \tau_t) \{ (1 - B) / (\zeta - B) \}$  where  $B$  is the amount of  $\zeta$  that is bound, and the subscript  $t$  indicates the value at full turgor. If the response is described by equation (1), then the relationship between  $\log \pi$  and  $\log \zeta$  will be linear. This was in fact so for the genotypes showing no evidence of osmoregulation, an example being *T. durum* (Fig. 3a). A value of  $\pi_t$  of -1.3 mPa was found by fitting a regression line to the data and then using this value in equation (1). The line predicted by this equation was reasonably close to the fitted line (Fig. 3a).

For genotypes showing evidence of osmoregulation, the

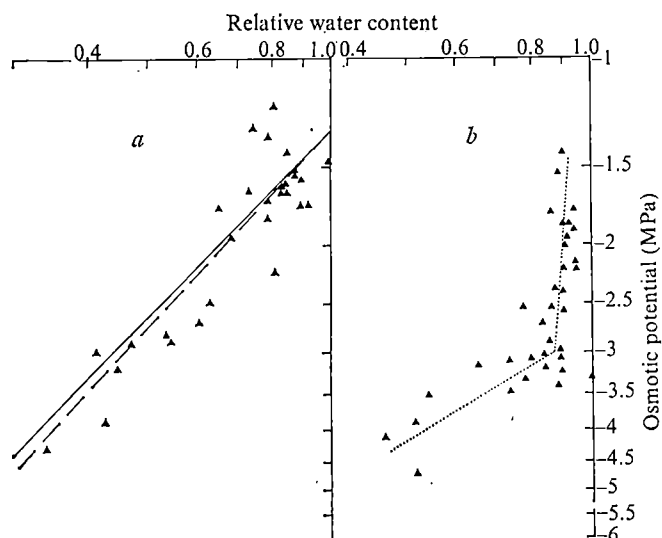


Fig. 3 Changes in the relative water content with osmotic potential for a, *T. durum* (AUS 2816) and b, *T. aestivum* (AUS 3850). The broken line is the line of best fit, the intact line that predicted by equation (1) and the dotted line an interpretation of Fig. 3b.

relationship seemed to consist of two distinct linear phases, as shown for *T. aestivum* (AUS 3850) in Fig. 3b. In the first, which covered the range of  $\pi$  from  $-1.5$  to  $-3.0$  MPa, there was virtually no change in  $\zeta$ . Since changes in  $\pi$  balanced changes in  $\Psi$  over this range (Fig. 2), the relative water content was conserved for changes in  $\Psi$  as well. At the end of this phase, there was a linear decline in  $\pi$  similar to that observed for plants of the type shown in Fig. 3a.

This pattern, being bi-modal, cannot be described by equation (1). Also, it is difficult to explain in terms of so called bound water, since the level would have to be about 0.9 in order to explain the first phase, with a rapid change to almost zero in order to explain the second. This would presumably require a sudden decrease in the amount of colloidal material in the leaf tissue. A more plausible explanation may be found if we assume that the solute content changes with decreases in  $\Psi$  up to the end of the first phase, that is, that osmoregulation occurs. At the end of this phase it seems that this process ceases and that  $\pi$  then declines with change in  $\Psi$  due to loss of water from the tissue and subsequent increase in the solute concentration, that is, according to equation (1). It is not clear from these results what limits solute accumulation, or why it should be present in some genotypes and not others. Indeed, there seems little point in speculating on these issues until more information has been obtained. It is, however, clear that the two types of response can occur in wheat, and that when osmoregulation occurs the turgor potential can be maintained over a substantial range of  $\Psi$ —up to  $-1.5$  MPa. More importantly, these results suggest that large genotypic variations in osmoregulation may exist, and that it may therefore be possible to improve the drought hardness of commercial wheat varieties by breeding for this characteristic.

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1. Hsiao, T. C. *A. Rev. Pl. Physiol.* 24, 519–570 (1973).
2. Greacen, E. L. & Oh, J. S. *Nature new Biol.* 235, 24–25 (1972).
3. Meyer, R. F. & Boyer, J. S. *Planta* 108, 77–87 (1972).
4. Ehlig, C. F. *Pl. Physiol.* 37, 288–290 (1962).
5. Slatyer, R. O. & Barrs, H. D. *UNESCO Arid Zone Res.* 25, 331–342 (1965).
6. Kassam, A. H. *New Phytol.* 72, 557–570 (1973).
7. Morgan, J. M. *Aust. J. Pl. Physiol.* 4, 75–86 (1977).
8. Turner, N. C. *Pl. Physiol.* 53, 360–365 (1974).
9. Warren-Wilson, J. *Aust. J. Biol. Sci.* 20, 359–367 (1967).

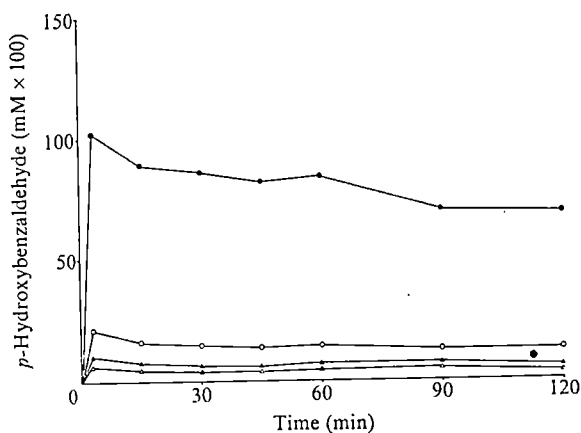
## Changes in release rates of cyanide in relation to palatability of *Sorghum* to insects

*SORGHUM* leaves contain the cyanoglycoside dhurrin located in the cell vacuole<sup>1</sup>, and when the tissue is crushed, hydrolysis of dhurrin by an enzyme system probably present in the cytoplasm<sup>1</sup> results in the release of HCN. Cyanogenesis is probably a mechanism to protect plants from being eaten by herbivores<sup>2,3</sup>. It is generally assumed that this protection is conferred by the toxic effects of cyanide but the mechanism has not been fully investigated and the cyanoglycosides themselves or the other end products of hydrolysis may deter feeding. The degree of liberation of HCN in leaves is dependent on the age and variety of plant, as well as on certain environmental factors<sup>4–7</sup>. Leaves of young *Sorghum* are often rejected at the first bite by the graminivorous locust, *Locusta migratoria*<sup>8</sup> but older *Sorghum* is eaten in large quantity. We show here that change in palatability is related to the rate at which HCN is released from the leaf at the time of biting.

Crystals of dhurrin (*p*-hydroxymandelonitrile- $\beta$ -D-glucoside) (melting point  $164^{\circ}$ – $168^{\circ}$  C), which liberated HCN when emulsin was added, were extracted<sup>9</sup> from 8-d-old *Sorghum bicolor* (variety 65D, from Botswana) grown in controlled conditions (18 h light,  $25^{\circ}$  C day,  $20^{\circ}$  C night). When dhurrin was tested for palatability by *Locusta* nymphs, by absorbing it on to sucrose-impregnated glass fibre papers which *Locusta* normally eat, it had no effect on feeding. The hydrolysis of dhurrin is stoichiometric<sup>10</sup> yielding, in addition to HCN, *p*-hydroxybenzaldehyde: this also had no effect on feeding. During the hydrolysis of dhurrin in freeze-dried *Sorghum* leaves, *p*-hydroxybenzaldehyde at first built up and then began to disappear from the test solution (compare ref. 10): the absorption maximum (in alkaline solution) shifted from 330 nm (*p*-hydroxybenzaldehyde) to 285 nm as the reaction proceeded probably because of accumulation of chelidonic acid<sup>11</sup>. Attempts to determine any antifeedant activity have proved inconclusive.

The release of HCN from crushed leaves was measured quantitatively by monitoring production of *p*-hydroxybenzaldehyde (Fig. 1). The amount released was very high in young plants and declined with age. On very young *Sorghum* insects

Fig. 1 Formation of *p*-hydroxybenzaldehyde from dhurrin present in *Sorghum* at different stages of development. Fresh tissue (0.5 g) of *Sorghum* 65D aged 8 d, 11.5 cm (●), 15 d, 16 cm (○), 30 d, 24 cm (▲) and 53 d, 40 cm (△) was crushed in distilled water (50 ml) and the mixture was aerated. Samples (5 ml) were withdrawn after 5 min and at 15-min intervals for 2 h, the reaction was stopped by the addition of acid and *p*-hydroxybenzaldehyde was extracted into ether, then into sodium bicarbonate and re-extracted into ether after acidification. The extracts were concentrated to dryness, the residue was redissolved in methanol and the absorbance at 285 nm due to *p*-hydroxybenzaldehyde was recorded.



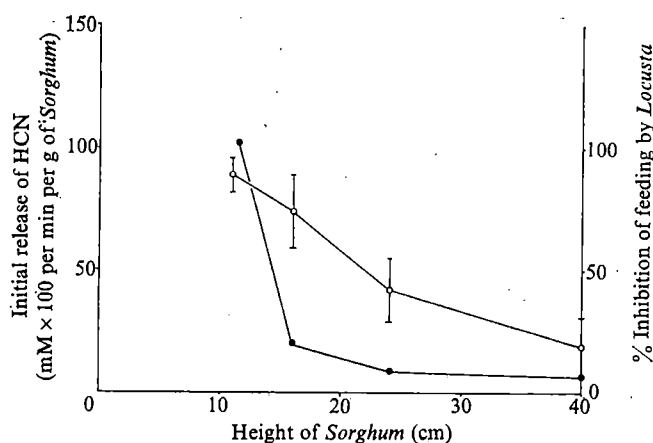


Fig. 2 Release of HCN from *Sorghum* at different stages of development in relation to feeding by *Locusta migratoria migratorioides*. Initial rates of release of HCN from *Sorghum* (●), as would occur on biting, could be calculated from data in Fig. 1 because the hydrolysis of dhurrin yields 1 mol of HCN and 1 mol of *p*-hydroxybenzaldehyde. Percentage inhibition of feeding (○) was found by comparing meal sizes of 5th instar nymphs on *Sorghum* of different ages, with meal sizes on mature, palatable leaves of *Poa annua* used as a control. (Standard deviations are indicated by vertical lines.)

reject the plant quickly; if HCN is the important deterrent it will be the amount released immediately on biting that is relevant rather than the capacity of the plant to produce HCN over a long period. The initial rate of HCN release was calculated for each plant age, and its relationship to the amount eaten in one meal is shown in Fig. 2.

The possibility of a rapid response to HCN was confirmed by introducing hydrocyanic acid into the mouth region of *Locusta*, by means of a previously fitted cannula, when the insect was feeding on a palatable grass. Concentrations as low as 0.01 mM were sufficient to inhibit feeding (Table 1). The amount of HCN initially released from young *Sorghum* (8-d-old) was calculated to be equivalent in concentration to a solution of between 0.5 mM and 1.0 mM hydrocyanic acid and thus would be effective as a deterrent.

Cyanoglycosides have been shown to have no effect on feeding by insects, nor to stimulate feeding at concentrations which occur naturally<sup>12</sup>. On the other hand, the release of HCN has been shown to protect bracken (*Pteridium aquilinum*)<sup>13</sup> and cassava (*Manihot esculenta*)<sup>14</sup> from insect attack. We have shown that *Sorghum* plants are best protected from insect attack when very young, and this protection is conferred by the HCN release on biting rather than by the cyanoglycoside itself or other products of its hydrolysis. Our work emphasises also

Table 1 Tests with hydrocyanic acid solution

Approx. concentration of HCN solution	No. of insects tested	Behaviour of 5th instar <i>Locusta</i> nymphs		
		No response	Feeding stops, but no backward movement	Feeding stops, also backward movement
1.0 M	5	0	0	5
0.1 M	5	0	0	5
0.01 M	10	0	4	6
1.0 mM	10	1	7	2
0.1 mM	10	4	6	0
0.01 mM	10	6	4	0
Water	10	10	0	0
Air	20	20	0	0

Different concentrations of hydrocyanic acid were introduced into the cibarial cavity of 5th instar nymphs through a chronically implanted cannula. Each insect had been deprived of food overnight and was tested during feeding on a palatable blade of grass. All tests were done during the first 3-min of feeding.

the importance of the initial rate of release of HCN when the insect bites.

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1. Saunders, J. A., Conn, E. E., Chin Ho Lin & Stocking, R. C. *Plant Physiol.* **59**, 647-652 (1977).
2. Jones, D. A. in *Taxonomy and Ecology* (ed. Heywood, V. H.) 213-242 (Academic, London, 1973).
3. Dement, W. A. & Mooney, H. A. *Oecologia* **15**, 65-76 (1974).
4. Dunstan, W. R. & Henry, T. A. *Phil. Trans. R. Soc., Lond.* **A199**, 399-410 (1902).
5. Williams, J. J. & West, R. M. *J. agric. Res.* **4**, 179-185 (1915).
6. James, J. H. & Gray, E. *Agron. J.* **67**, 82-84 (1975).
7. Hogg, P. G. & Ahlgren, H. L. *J. agric. Res.* **67**, 195-210 (1963).
8. Bernays, E. A., Chapman, R. F., Horsey, J. & Leather, E. M. *Bull. ent. Res.* **64**, 413-420 (1974).
9. Towers, G. H. N., McInnes, A. G. & Neish, A. C. *Tetrahedron* **20**, 71-77 (1964).
10. Akazawa, T., Miljanich, P. & Conn, E. E. *Plant Physiol.* **35**, 535-538 (1960).
11. Bough, W. A. & Gander, J. E. *Phytochemistry* **11**, 209-213 (1972).
12. Nayer, J. K. & Fraenkel, G. *Ann. ent. Soc. Am.* **56**, 174-176 (1963).
13. Cooper-Driver, G. A. & Swain, T. *Nature* **260**, 604 (1976).
14. Bernays, E. A., Chapman, R. F., McCaffery, A., Modder, W. & Leather, E. M. *Bull. ent. Res.* (in the press).

## New approach to pollen culture

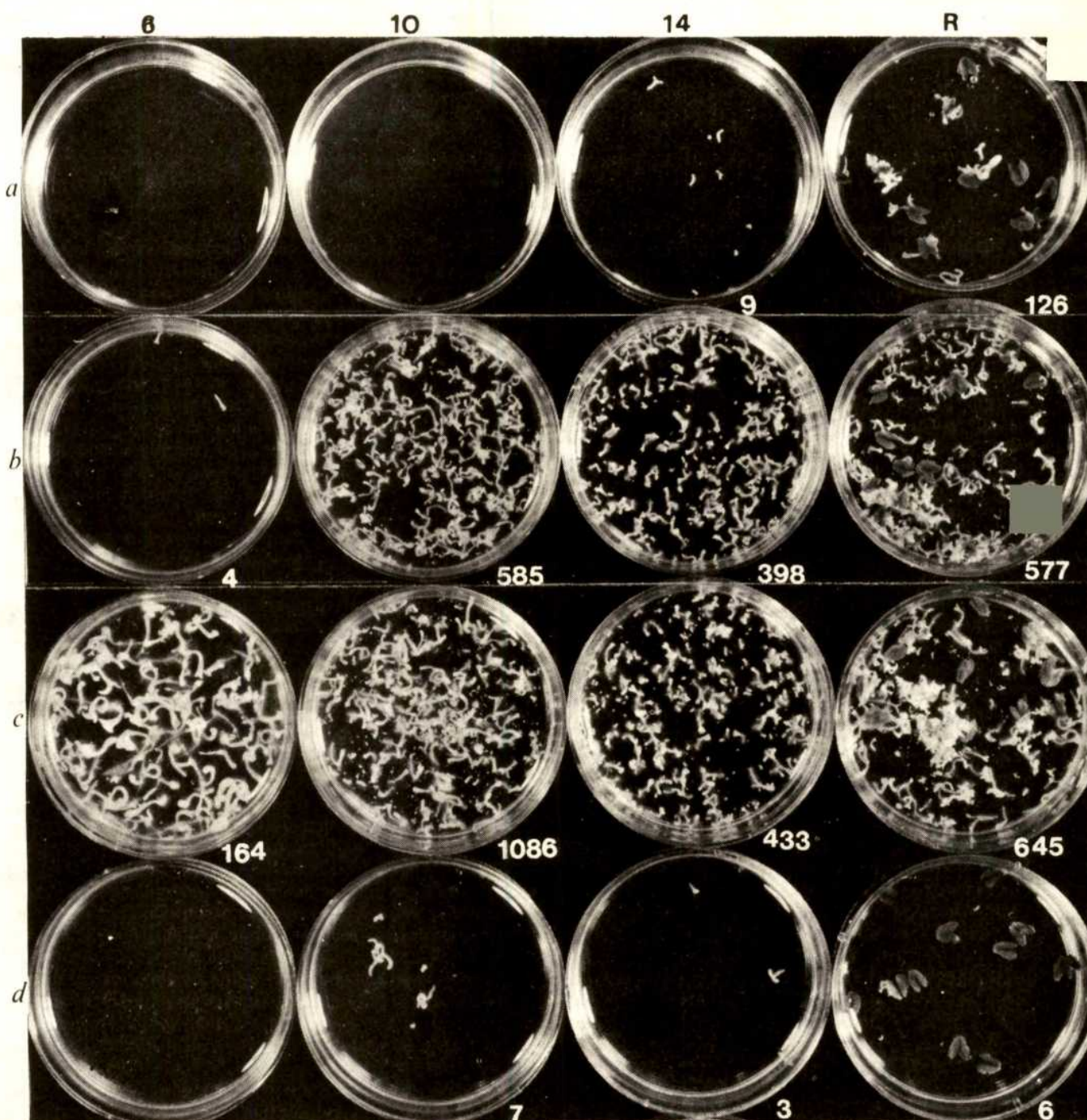
RESEARCH on pollen and anther culture is important for genetic and mutation studies, and has many potential applications in plant breeding and crop improvement. In our experience, the technique of pollen culture developed by Nitsch and co-workers<sup>1,2</sup>, though successful with a wide range of species<sup>3</sup>, is much more difficult than anther culture and involves procedures which are unreliable and inefficient. Furthermore, plant yields are generally lower than when anther culture is used. We describe here an alternative approach which retains the simplicity and reliability of anther culture while eliminating the need for either mechanical disruption<sup>1,2</sup> or surgical manipulation<sup>4</sup> of anthers.

Our approach exploits the fact that, in many species, anthers dehiscence soon after inoculation, and if they are floated on shallow layers of liquid medium in plastic Petri dishes, pollen is 'shed' into the medium. This shed pollen will continue to grow if the anthers are removed from the dishes. Shedding continues throughout the culture period; hence, by frequent transfer of anthers to fresh medium a sequence of pollen cultures can be obtained from the same batch of anthers.

Examples of cultures of pollen shed from anthers, instead of mechanically isolated from them, are shown in Fig. 1 for *Nicotiana tabacum*. Illustrated is a series of cultures initiated with anthers of increasing age (Fig. 1 a-d) and in which the anthers have been transferred into fresh medium at 6, 10 and 14 d; in each case dishes were resealed after transfer of the anthers and reincubated together with a fourth dish containing the residual anthers (R). In cultures initiated at the unicellular pollen stage (microspores) (Fig. 1a), anthers do not open to any great extent and little shed pollen develops over the first 14 d. With increase in anther age, however, the time to anther opening decreases whereas embryo yields increase, and shed fractions develop at progressively earlier stages of culture (Fig. 1b, c). With anthers containing wholly young bicellular pollen grains (microgametophytes) (Fig. 1c), shed fractions develop after 0-6 d. Pollen is shed most rapidly in cultures of still older anthers (Fig. 1d) but as is now well known the embryogenic potential of older anthers is limited.

Chilling of buds before excision and culture of the anthers<sup>1</sup> is another important requirement for early dehiscence of tobacco anthers. We find periods of 12 d and longer at 7-8 °C optimal for this species. To minimise water loss during cold treatment, we store the buds in polythene bags wrapped in thin aluminium foil, a procedure which also maintains a high percentage of viable pollen (as assessed by fluorescein diacetate<sup>5</sup>). Illumination of cultures for the whole of the culture period has





**Fig. 1** *Nicotiana tabacum* cv. White Burley. Effect of developmental stage on anther dehiscence and liberation of pollen into liquid culture medium. *a*, Unicellular pollen stage (corolla length 13–15 mm). *b*, First pollen division stage (17–19 mm). *c*, Early bicellular pollen stage (21–23 mm). *d*, Bicellular stage just before deposition of starch (28–30 mm). In all cases, buds were stored at 7–8 °C for 12 d before excision and inoculation of the anthers. At inoculation, 12 anthers from three buds of similar stage were floated on the surface of 5 ml of liquid A medium. They were transferred to fresh A medium after 6, 10 and 14 d. All dishes were sealed with Parafilm and incubated at 28 °C in darkness for the first 14 d. After 14 d, they were transferred to light (Grolux tubes, 12-h day, 500 lx) at 25 °C. Embryo counts were made under a binocular after 35 d while the embryos were still small and separate from each other. Chromosome counts made on 10 embryos from each dish in (*b*) and (*c*) were all haploid. R, dish containing the residual anthers. A medium, major salts (half strength) and Fe-EDTA (full strength) of Murashige and Skoog<sup>10</sup> plus sucrose 2%; pH 5.5.

so far proved detrimental as has shaking them on a horizontal shaker at 130 r.p.m. Our best results have been obtained with stationary cultures incubated in darkness for 14 d at 28 °C before transfer to light at 25 °C.

The performance of tobacco pollen 'shed' from anthers initially at the mitotic to early bicellular stages and that of pollen mechanically isolated from the anthers is indicated by the data of Table 1. Embryo yields are given for fractions shed into a simple nutrient medium (A medium) and fractions of isolated pollen mounted in either A medium or A medium supplemented with glutamine, serine and *myo*-inositol (AGSI

medium) as recommended by Nitsch<sup>2</sup>. If we consider the data for the 8-d chilled material (which show the highest yields), it will be seen that the isolated pollen responds to AGSI medium (but not A medium) after preculture of the anthers for 3 d or longer, thus confirming Nitsch's findings. But in none of the pollen isolates is the yield as great as in the anther-culture controls. Shed fractions start to develop in the 8-d chilled material after 7 d of culture and by 9 d the yield exceeds that in any of the isolated fractions. The pollen is shed into A medium and the fact that it will then grow in the absence of the anther tissues implies a conditioning of the medium by the



**Table 1** Comparison of embryo yields in cultures of anthers, isolated pollen and shed pollen of *Nicotiana tabacum* cv. White Burley

Pollen fraction	Time of chill (d)	Preculture before isolation of pollen (d)										No isolation anther-culture controls	
		1		3		5		7		9		35	
Isolated	0	I	II	I	II	I	II	I	II	I	II	I	II
	4	0	0	0	0	0	0	0	3	1	5	22	35
	8	0	0	0	3	0	9	0	0	0	30	37	68
Shed	0	0	0	0	71	0	35	2	34	2	63	120	116
	4	0	0	0	0	0	0	0	0	1	8		
	8	0	0	0	0	0	0	34	90				

Mean embryo yields per anther from two experiments. I, Pollen cultured in A medium (see Fig. 1). II, Pollen cultured in AGSI medium (A medium plus L-glutamine 800 mg<sup>-1</sup>, L-serine 100 mg<sup>-1</sup> and myo-inositol 5,000 mg<sup>-1</sup>). In each experiment, three groups of 25 buds were harvested at the mitotic to early bicellular pollen stage (corolla length 17–23 mm). One group was dissected without cold treatment, the other two groups were stored at 7–8 °C for 4 and 8 d respectively before dissection. After dissection the five anthers from each bud were distributed singly into 5-cm Petri dishes containing 5 ml of liquid A medium, to give five replicates each of 25 anthers. One anther was then removed from each dish and floated on 5 ml of A medium, and a second anther from each dish was floated on AGSI medium (anther-culture controls). At 1, 3, 5, 7 and 9 d, one dish was taken from each group of five. Dead anthers (dark brown) were rejected; the remainder (about 20) were then removed and the dish resealed ('shed' fraction). The anthers removed were gently ground in a Potter glass homogeniser. Homogenates were filtered through 100-µm nylon and the filtrate centrifuged for 4 min at 100g. The supernatant was discarded. The pellet was resuspended in 10 ml of A medium, divided into two aliquots, recentrifuged and the supernatants again discarded. One pellet was inoculated into 5 ml A medium, the other into 5 ml of AGSI medium (isolated pollen). All dishes were sealed with Parafilm and incubated at 28 °C in darkness for the first 14 d, thereafter at 25 °C in Grolux light (12-h day, 500 lx). Embryos counted after 35 d. All buds were harvested within 2 weeks from the same batch of glasshouse plants grown through November to January under supplementary illumination.

anther tissues. Tapetum has long been thought to have a nutritive function and it is thus possible that conditioning of the medium stems from materials released from degenerating tapetal cells. Conditioning is a continuous process, and it is evident from the serial cultures of Fig. 1 that contact between anthers and medium for as little as 4 d is sufficient to support growth of large numbers of embryos. Evidence of medium-conditioning by anther tissues has also come from work on pollen isolated by surgical manipulation of anthers<sup>4</sup>.

In its simplicity the new approach to pollen culture has decided advantages for both applied and fundamental research. For mutation studies, we see no intrinsic objection to the use of fractions shed after between 0 and 6 d instead of fractions mechanically isolated from anthers at, say, 3–4 d. Besides eliminating possible deleterious effects of the homogenised anther tissues, the new procedure leaves the tissues intact, making them available for further experimentation and analysis. The new procedure is particularly attractive to large-scale manipulation of species possessing minute anthers, as in some of the cereals, and we have already found that, in barley, pollen calluses emerging from floating anthers are shed into the medium. Encouraging results have also been obtained in other Solanaceous species. Scrupulous attention must be given to anther-staging. For instance, in *N. knightiana*, anthers in the immediate post-first-division stage of the pollen are crucial, whereas in *Hyoscyamus niger*, the immediate pre-division stage seems to be the most productive. The success of the new procedure is undoubtedly due to the use of liquid, instead of agar, media. Advantages of liquid media, first reported for *N. tabacum* by M. Devreux (at the 1974 Haploid Conference held in Guelph), are now being realised by other workers and exploited in different species<sup>6–9</sup>.

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1. Nitsch, C. & Norreel, B. C. r. *Acad. Sci. Paris* 276, 303–306 (1973).
2. Nitsch, C. in *Haploids in Higher Plants: Advances and Potential* (ed. Kasha, K. J.) 123–135 (1974).
3. Sunderland, N. & Roberts, M. *John Innes Inst. a. Rep.* 61–65 (1976).
4. Wernicke, W. & Kohlenbach, H. W. Z. *Pflanzenphysiol.* 81, 330–340 (1977).
5. Heslop-Harrison, J. & Heslop-Harrison, Y. *Stain Technol.* 45, 115–120 (1970).

6. Devreux, M., Laneri, U. & de Martinis, P. *Giorn. Bot. Ital.* 109, 335–349 (1975).
7. Dunwell, J. M. thesis Univ. East Anglia (1975).
8. Wernicke, W. & Kohlenbach, H. W. Z. *Pflanzenphysiol.* 77, 89–93 (1975).
9. Wernicke, W. & Kohlenbach, H. W. Z. *Pflanzenphysiol.* 79, 189–198 (1976).
10. Murashige, T. & Skoog, F. *Physiologia Pl.* 15, 473–497 (1962).

## Reproductive roles in the simultaneous hermaphrodite *Aplysia dactylomela*

THE opisthobranch gastropod *Aplysia* is a simultaneous hermaphrodite with internal fertilisation. The functional morphology of the reproductive system<sup>1–4</sup> has been well studied and oviposition is known to be under some hormonal control<sup>5–7</sup>. Adults may assume the role of sperm donor or recipient, or both at the same time. Recent discussions of the evolutionary significance of simultaneous hermaphroditism<sup>2,8–14</sup> as well as considerations of the relative roles of sperm donors and recipients in selecting partners<sup>15,16</sup>, have suggested that *Aplysia* offers an opportunity to investigate the processes involved in the assumption of different sex roles in the same animal. Accordingly we analysed data collected several years ago during other field studies of *Aplysia dactylomela*<sup>17,18</sup>. We found that most animals were as likely to be sperm donors as recipients during different copulations, and there was a significant relationship between frequency of copulation and number of different partners in the total population studied. A few individuals, however, were found to copulate with fewer partners than sperm recipients.

We used *A. dactylomela* maintained in the Lerner Marine Laboratory, Bimini, Bahamas. Animals were collected during the day from the thalassia flats of the Bimini lagoon and placed in 1,000-l concrete tanks supplied with water from the ocean. The behaviour of groups of 5–10 animals was observed every 30 min between 0530 and 1130 and between 1200 and 2300 for 3 weeks; each group was observed 3–22 times. Individuals were identified by a colour-coded stainless steel safety pin inserted through the parapodium. Time of day had been shown to influence the probability that the animals would be found alone or copulating in their natural habitat<sup>18</sup>; we found that the same was true of animals maintained in laboratory conditions. Copulations occurred significantly more often in the morning ( $P < 0.05$ ,  $\chi^2$  test<sup>19</sup>). We also confirmed that time of day did not significantly relate to the number of times animals were found alone, or in contact.

As Fig. 1 shows, most individuals were observed copulating

between three and seven times. To facilitate statistical analysis of the roles assumed in copulation, we selected animals which copulated five times or more. This enabled us to use the binomial test with an associated probability of 0.06 or less<sup>19</sup>. By this criterion, we had 26 individuals which copulated five or more times. Of these, 17 did not copulate consistently in one or the other role, four were sperm recipients more often than donors, and five were sperm donors more often than recipients.

Thus our sample behaved as might be expected of simultaneous hermaphrodites in that the probability of finding individuals which consistently assumed one or another sexual role was relatively low. But we wanted to know whether the nine consistent animals represented a true behavioural subsample. It seemed that the three behavioural 'types' might be distributed differentially with respect to frequency of copulation.

As Fig. 2 shows, the number of times an individual copulated was positively correlated with the number of different partners ( $r_s = 0.86$ ,  $P < 0.01$ ). Animals which consistently assumed the same sex role were in a different part of the distribution from the others. The group medians for number of different partners (3.5) and number of observed copulations (5.5) segregated seven of these nine animals in the upper right quadrant of the graph. Thus all the consistent sperm recipients and three of the five consistent sperm donors copulated frequently and with many different partners. Further comparisons suggested that sperm recipients copulate with more partners than do sperm donors (Mann-Whitney  $U$  test,  $U = 3$ ,  $P = 0.056$ ).

This latter finding implies some degree of interindividual organisation that may be significant for adaptations of the species. We cannot eliminate the possibility that the differences in donor or recipient roles are due to sampling error; although individuals were collected at the same time, they may have been at different developmental stages or in different reproductive states. But if some part of any population behaves consistently as donors or recipients for a significant time, selection could act on them when pressures favour or disfavour behaviourally dimorphic individuals in reproduction.

We saw egg-laying animals simultaneously acting as sperm recipients but not as sperm donors. Genetic variability might therefore be increased in the least 'expensive' way if the sperm donor spends energy searching for sperm recipients, while the recipient spends energy on oviposition. Even though the whole population 'spends' equally on female and male functions, the differential behaviour patterns of a small group of individuals may well be significant for selection processes<sup>9,20</sup>. Thompson and Bebbington<sup>4</sup> suggested that *Aplysia* sperm are activated only after transfer to the recipient. Further work is needed to determine which sperm are more likely to fertilise the eggs—the more recently donated sperm, or that from previous copulations which has been stored for some time. Competition between sperm, such as that described for insects<sup>21</sup>, may be a mechanism in *Aplysia* by which more successful reproductive behaviours are selected.

Charnov and Bull<sup>11</sup> argued that in sessile simultaneous hermaphrodites selection favours the ability to alter the ratio

Fig. 1 Copulation frequencies observed in 37 individual *Aplysia dactylomela* maintained in tanks in the Lerner Marine Laboratory.

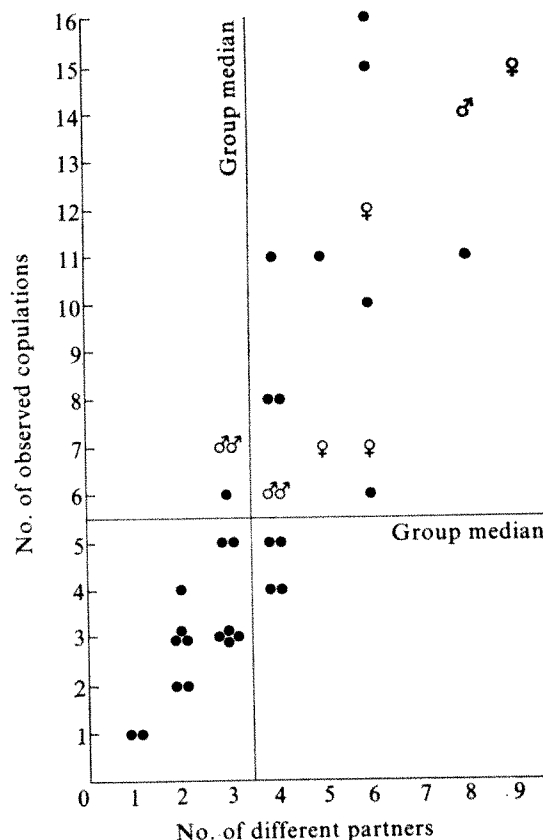
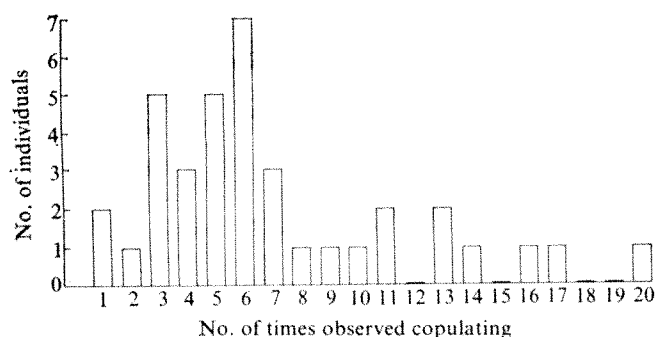


Fig. 2 Relationship between frequency of observed copulation and number of partners in *A. dactylomela*. ♀, sperm recipient; ♂, sperm donor; ●, one animal.

of sperm to ova in response to changing environmental conditions. It is possible that in the highly mobile *Aplysia*, the quantity and function of stored sperm may be regulated by the organisation of copulatory interactions.

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1. Bebbington, A. & Thompson, T. E. *Malacology* 9, 253 (1969).
2. Ghiselin, M. T. *Malacology* 3, 327-378 (1965).
3. Thompson, T. E. *The Biology of Opisthobranch Molluscs* (Ray Society, London, 1976).
4. Thompson, T. E. & Bebbington, A. *Malacology* 7, 347-380 (1969).
5. Arch, S. & Smock, T. *Behav. Biol.* 19, 45-54 (1977).
6. Kupfermann, I. *J. Neurophysiol.* 33, 877-881 (1970).
7. Coggeshall, R. E. *Am. Zool.* 12, 521-523 (1972).
8. Ghiselin, M. T. *Q. Rev. Biol.* 44, 189-208 (1969).
9. Williams, G. C. *Sex and Evolution* (Princeton University Press, 1975).
10. Williams, G. C. & Mitton, J. B. *J. theoret. Biol.* 39, 545-554 (1973).
11. Charnov, E. L. & Bull, J. *Nature* 266, 828-830 (1977).
12. Maynard Smith, J. in *Group Selection* (ed. Williams, G. C.) (Aldine-Atherton, Chicago, 1971).
13. Ghiselin, M. T. *Nature* 258, 32 (1975).
14. Charnov, E. L., Maynard Smith, J. & Bull, J. *Nature* 263, 125-126 (1976).
15. Zahavi, A. *J. theoret. Biol.* 53, 205-214 (1975).
16. Smith, J. M. *J. theoret. Biol.* 57, 239-243 (1976).
17. Tobach, E., Gold, P. & Zeigler, A. *Veliger* 8, 16-18 (1965).
18. Lederhendler, I., Bell, I. & Tobach, E. *Veliger* 17, 347-353 (1975).
19. Siegel, S. *Nonparametric Statistics* (McGraw-Hill, New York, 1956).
20. Hunt, Jr G. L. & Hunt, M. W. *Science* 196, 1466-1467 (1977).
21. Parker, G. A. *Biol. Rev.* 45, 525-567 (1970).



## Gametogenesis in culture by gametocytes of *Plasmodium falciparum*

CONTINUOUS culture of human malaria parasites, *Plasmodium falciparum*, in human red blood cells was first reported by Trager and Jensen<sup>1</sup> and subsequently by Haynes and co-workers<sup>2</sup>. The culture system described by Trager and Jensen supports the asexual multiplication of *P. falciparum* and the formation of gametocytes (the precursors of the gametes). Gamete formation by cultured *P. falciparum*, without which the parasites are unable to infect mosquitoes, has not been previously described. We report here that gametocytes of *P. falciparum* grown from parasites maintained by continuous culture *in vitro* are able to develop to the point at which they can be stimulated to undergo gametogenesis (exflagellation).

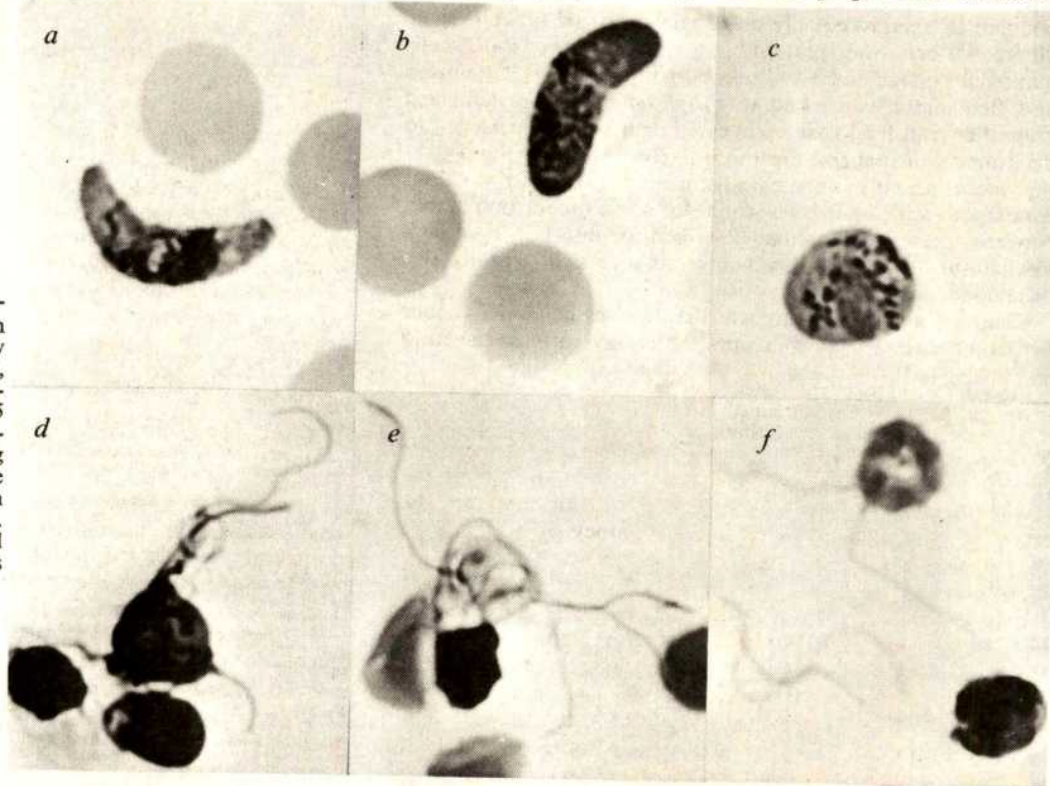
The isolate of *P. falciparum* used in this study was adapted to culture on 4 January 1977 from the blood of an individual who had become infected during a visit to Zaire from September to December 1976. This isolate, designated the Z strain, was obtained with the kind cooperation of Dr David Wyler. The parasites were adapted to growth in red cells of blood type A. Infected cells were suspended in culture medium at a haematocrit of 6%, placed in 30 ml tissue culture flasks (Corning no. 25100) with a 25 cm<sup>2</sup> culture surface area and incubated at 37 °C. Each flask contained 5.0 ml of cell culture. The medium used in routine culture was that described by Trager and Jensen<sup>1</sup> and consisted of RPMI 1640 powdered medium supplemented with HEPES buffer (25 mM) and 0.2% NaHCO<sub>3</sub> with 10% human serum type AB. Each day the culture medium was removed from the surface of the settled cells and replaced with fresh medium. Flasks were gassed with a mixture containing 6% O<sub>2</sub>, 3% CO<sub>2</sub> and 91% N<sub>2</sub>. During routine maintenance the parasite cultures were diluted with fresh red cells every 4–8 d. Parasite densities usually rose to between 1% and 2% of blood cells infected.

Smalley<sup>3</sup> has reported that gametocytes in non-growing

cultures of *P. falciparum* require 10 d to develop from a merozoite to a stage approaching morphological maturity. In the present study certain cultures were maintained for 2–3 weeks without dilution to allow gametocytes to reach full maturity. In cultures maintained in this way immature gametocytes were usually present after 1 week. By 2 weeks numerous gametocytes were usually present including many with the morphological appearance of maturity (Fig. 1).

In one culture vessel, set up on 25 February 1977 when the parasites had been maintained in continuous culture for almost 7 weeks, the regular medium was replaced 15 d later with one containing 50% serum instead of the usual 10%. The culture was maintained in this medium thereafter. Eight days after substituting with the new medium the gametocytes density was 0.6%. At this stage almost 50% of the gametocytes were apparently mature by morphological criteria on blood smears stained with Giesma's stain. Mature gametocytes of both sexes were crescent shaped cells with rounded ends and pigment granules concentrated near or over the nucleus near the centre of the crescent (Fig. 1). Male and female gametocytes were distinguished from one another by their staining properties. The cytoplasm of female gametocytes stained blue while that of males stained orange-pink and was not easily distinguished from the nucleus. About 35% of all gametocytes were morphologically mature females and about 10% were morphologically mature males. The remaining gametocytes were in various stages of development.

Gametocytes from this culture were stimulated to undergo gametogenesis by the following method. On the eighth day after substituting with medium containing 50% serum the cells were evenly resuspended in their own culture medium and 0.3 ml of the suspension dispensed in a serum tube into 3.0 ml of foetal bovine serum (FBS) adjusted to pH 8 with 1.5% NaHCO<sub>3</sub>. The cells were sedimented by centrifugation for 30 s; most of the supernatant was removed and the packed cells were resuspended in a minimum volume of the remaining supernatant fluid. One drop of this cell suspension was placed on a microscope slide, a cover slip placed on top and the preparation examined



**Fig. 1** Gametocytes and gametogenesis of *P. falciparum* in culture. *a*, Morphologically mature macrogametocyte; *b*, morphologically mature microgametocyte; *c*, macrogamete 25 min after initiation of gametogenesis; *d*, *e*, *f*, exflagellating microgametocytes showing the release of microgametes 25 min after initiating gametogenesis. All preparations were methanol fixed and stained with Giesma's stain.



under a microscope. Between 5 and 10 min after the cells had been resuspended in FBS extensive rounding of gametocytes was observed. At 15 min the first exflagellation was seen (Fig. 1). During subsequent observation, up to 45 min, about one exflagellating male gametocyte was observed per 10,000 red blood cells. This represents about one exflagellating male gametocyte for every five male gametocytes judged to be mature by morphological criteria. Over 50% of female gametocytes judged morphologically mature were found to have rounded up to form female gametocytes when examined on blood smears stained with Giesma's stain.

When gametocytes were examined on a slide in culture medium without washing in FBS at pH 8.2, no evidence of gametogenesis was seen during 45 min of observation under the microscope.

Two days later the experiment was repeated on the same culture using human serum adjusted to pH 8.2 instead of FBS as the exflagellating medium. Gametogenesis (exflagellation) was again successfully initiated. The same method for stimulating exflagellation was applied to three other cultures. These were only 2 weeks old and had been maintained throughout in medium containing 10% human serum. In two of these cultures exflagellation was successfully initiated although the frequency of exflagellation events was less than that observed in the 3-week-old culture.

It is clear from these results that gametocytes produced by the Trager-Jensen system of malaria cultures can develop to a point at which they are capable of undergoing gametogenesis. At this stage our observations are not adequate to define the precise conditions required to achieve this reproducibly. Prolonged maintenance of cultures for at least 2 weeks is probably important to allow gametocytes to reach full maturity. Transfer to a medium containing high serum may also be important in this respect. It is well known that certain malaria parasites (for example, rodent malaras) lose their ability to form gametocytes after prolonged blood passage in laboratory hosts without mosquito transmission. An analogous situation may apply to *P. falciparum* maintained for prolonged periods in culture. If this is true it may be significant that we achieved our results with a line of parasites recently isolated from a natural infection.

Account should also be taken of the fact that the demonstration of exflagellation *in vitro* depends on the use of appropriate methods for stimulating gametogenesis. In studies with other species of malaria parasites it has been found that bicarbonate-containing media at pH 8.0, most effectively animal sera, are excellent for this purpose<sup>4,5</sup>. At pH values below 7.7 and in media from which bicarbonate is absent exflagellation usually does not take place. In the conditions used to culture *P. falciparum* the Trager-Jensen culture medium is maintained at a pH of 7.3 to 7.4. The presence of a powerful buffer (25 mM HEPES) in this medium may prevent the pH from rising sufficiently rapidly to initiate gametogenesis during equilibration with the atmosphere. Thus after exposing a drop of the cell suspension to the atmosphere, the method commonly used to initiate exflagellation, we were unable to observe gametogenesis by gametocytes in their original culture medium. By washing and resuspending cultured gametocytes in bicarbonate supplemented sera at pH 8.0, exflagellation was readily demonstrated.

Our observations demonstrate that cultured blood stages of *P. falciparum* are capable of generating gametocytes able to undergo gametogenesis. Although the conditions which would enable this to be carried out on a regular basis are not completely defined there is good reason to expect that cultured blood stages of *P. falciparum* may be used to generate the gametes and mosquito stages of this parasite.

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- <sup>1</sup> Trager, W. & Jensen, J. B. *Science* **193**, 673-675 (1976).
- <sup>2</sup> Haynes, J. D., Diggs, C. L., Hines, F. A. & Desjardins, R. E. *Nature* **263**, 767-769 (1976).
- <sup>3</sup> Smalley, M. E. *Nature* **264**, 271-272 (1976).
- <sup>4</sup> Bishop, A. & McConnachie, E. W. *Parasitology* **50**, 431-448 (1960).
- <sup>5</sup> Carter, R. & Nijhout, M. M. *Science* **195**, 407-409 (1977).

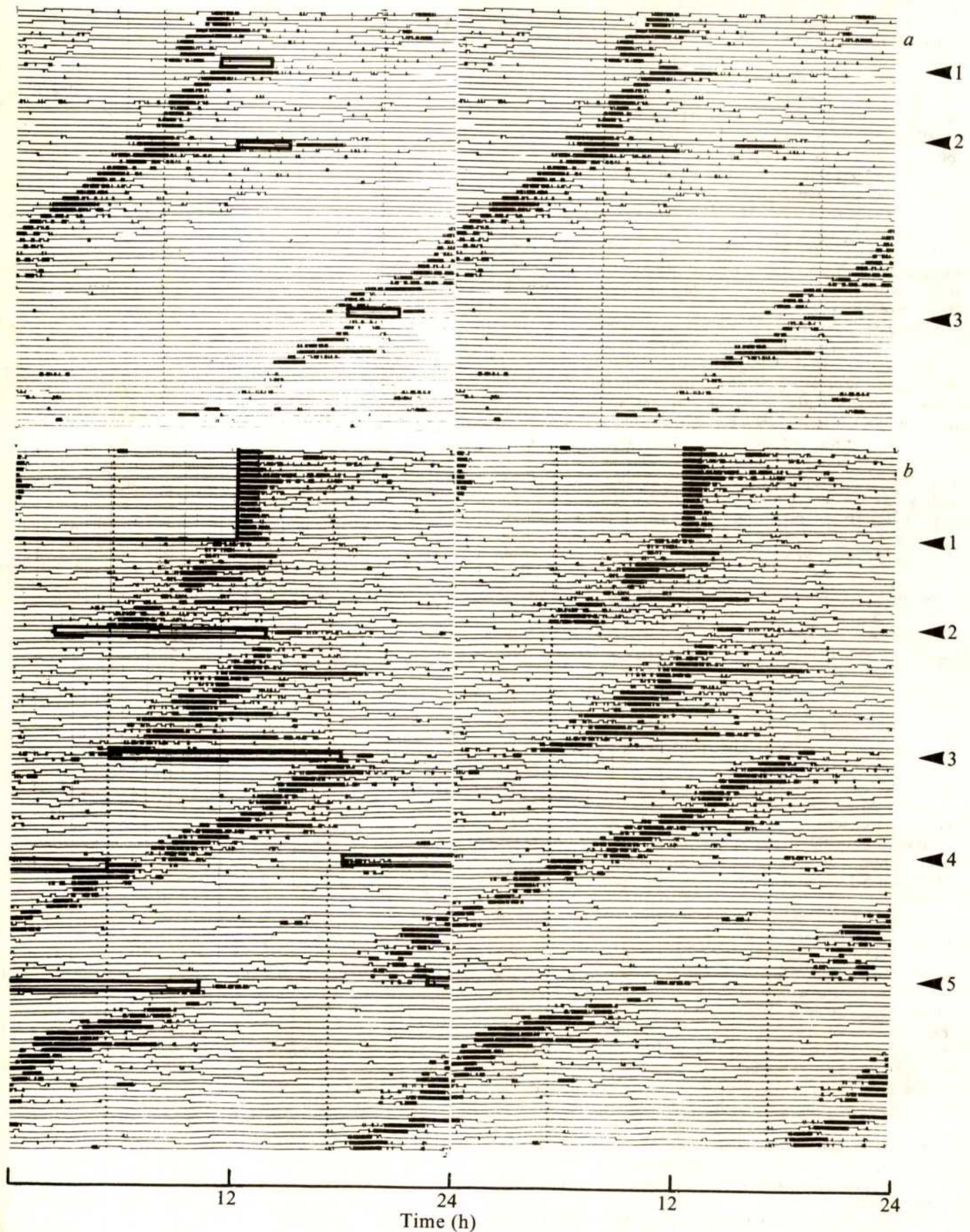
## 'Strong' phase response curve for the circadian rhythm of locomotor activity in a cockroach (*Nauphoeta cinerea*)

ANALYSIS of entrainment in circadian systems depends on a knowledge of the phase resetting effects of the light (or temperature) pulses perturbing the circadian oscillation. Thus when a light pulse of a particular intensity and/or duration falls at certain phases of the free-running oscillation it may generate phase-advances ( $+\Delta\phi$ ) in subsequent activity; when it falls at other phases it may generate phase-delays ( $-\Delta\phi$ ). A plot of such phase changes, both magnitude and sign, as a function of the phase of the oscillation so perturbed, is called a phase response curve (PRC) (refs 1, 2). In a systematic study of the resetting effects of light pulses on the rhythm of pupal eclosion in *Drosophila pseudoobscura*, Winfree<sup>3</sup> demonstrated two topologically different types of PRC, depending on the strength of the signal. When light pulses were 'weak' ( $< 50$  s blue light,  $10 \mu\text{W cm}^{-2}$ ) phase shifts were small. When pulses were 'strong' ( $> 50$  s) phase shifts became abruptly larger, up to 10-12 h. Because of the way Winfree's data were graphically presented, weak resetting curves were called type 1 and strong curves type 0. An abrupt change from type 1 to type 0 has been observed only in circadian systems which control physiological events such as pupal eclosion (refs 1-3 and D.S. in preparation) and oviposition<sup>5</sup> in populations of insects. Rhythms of locomotor activity in single animals, on the other hand, have only shown low amplitude type 1 curves<sup>6,7</sup>. Entrainment in 'physiological' rhythms is in most cases mediated by extraoptic photoreceptors and a hormonal output<sup>8</sup>. 'Behavioural' rhythms, on the other hand, most frequently involve entrainment by way of the organised photoreceptors<sup>9,10</sup> and an electrical (neural) output<sup>10-12</sup>. Truman used these differences to distinguish two types of biological clock<sup>13</sup>. Here, however, we report PRCs which switch from type 1 to type 0 in the locomotor activity rhythm of a cockroach *Nauphoeta cinerea*, which might from earlier literature have been expected to show only type 1 resetting.

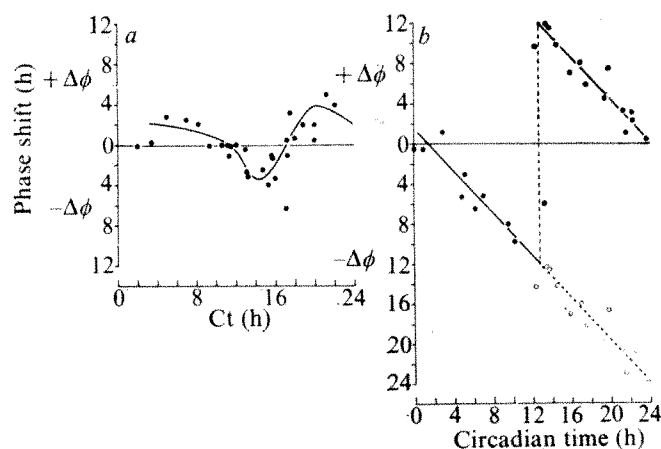
Newly-emerged males of *N. cinerea* were housed singly in small Perspex running wheels, in light-tight boxes at  $25 \pm 0.5^\circ\text{C}$ . Each revolution of the running wheel caused four closures of a magnetic proximity switch, and the activity of the cockroach was thus recorded by an Esterline-Angus event recorder. For each insect, activity records for successive 24-h periods were pasted down in chronological order on poster boards (Fig. 1). Records of locomotor activity were then photographed and 'double-plotted' to enhance any rhythmic features.

Insects were initially entrained to a light cycle of 12 h of light and 12 h dark (LD 12:12) for 10-14 d. The lights were then turned off and the activity rhythms allowed to free-run in continuous darkness. Measurements of free-running periods ( $\tau$ ) were made by eye-fitting a line to the onsets of activity (Fig. 1), the slope of the line indicating  $\tau$ . Cockroaches in free-running conditions were then perturbed by single 3 h or 12 h light pulses ( $240 \mu\text{W cm}^{-2}$ ), with





**Fig. 1** Locomotor activity rhythms of two males of the cockroach *Nauphoeta cinerea*. *a*, 'Free-running' in continuous darkness (DD) and perturbed by single 3-h pulses of white light ( $240 \mu\text{W cm}^{-2}$ ) at: 1, circadian time (Ct) 14.7 ( $-\Delta\phi 2.5 \text{ h}$ ); 2, Ct 17.1 ( $+\Delta\phi 0.5 \text{ h}$ ); 3, Ct 13.3 ( $-\Delta\phi 3.1 \text{ h}$ ). *b*, Initially entrained to LD 12:12, then: 1, 'free-running' in DD and perturbed by single 12-h pulses of white light ( $240 \mu\text{W cm}^{-2}$ ) at: 2, Ct 10.2 ( $-\Delta\phi 9.8 \text{ h}$ ); 3, Ct 13.5 ( $+\Delta\phi 11.8 \text{ h}$ ); 4, Ct 22.3 ( $+\Delta\phi 2.2 \text{ h}$ ); 5, Ct 15.9 ( $+\Delta\phi 7.0 \text{ h}$ ). Activity records are 'double-plotted' to enhance rhythmic features. Light treatments are shown as open boxes in the left-hand panel.  $-\Delta\phi$ , delay phase shift (h);  $+\Delta\phi$ , advance phase shift (h).



**Fig. 2** Phase response curves for 3-h (a) and 12-h (b) pulses of white light ( $240 \mu\text{W cm}^{-2}$ ) in the cockroach *Nauphoeta cinerea*. For 3-h pulses the response curve is of the 'weak' type 1 with small phase changes (4–5 h). For 12-h pulses the response curve is of the 'strong' type 0, drawn either as a 'saw-tooth' curve showing phase delays and phase advances (up to 12 h), or as a monotonic straight line with all phase changes plotted as delays ( $y = -1.06572x + 1.6640$ ;  $r = 0.96$ ;  $P < 0.001$ ).

the resetting pulses being timed to occur at all phases (circadian times, Ct) of the insects' endogenous circadian period. Phase shifts, in hours, were measured as displacements in activity onsets before and after the pulse.

The phase response curve for 3-h pulses of white light ( $240 \mu\text{W cm}^{-2}$ ) (Fig. 2a) was of the low 'amplitude' type 1 with phase delays early in the 'subjective night' (Ct 12–18) and phase advances late in the 'subjective night' (Ct 18–24). Maximum phase changes were in the order of 4–5 h. Phase advances were distinguished from phase delays, not only by their final steady state in relation to the activity onset before the pulse, but also on the criterion of reaching that steady state through a series of non-steady-state or transient cycles, which are evident for advances but not delays<sup>1</sup>. For 12-h pulses phase changes were much larger, the resultant response curve (Fig. 2b) either being represented by a high amplitude saw-tooth with a  $360^\circ$  'dataless' change of phase between delays and advances at about Ct 13 or, more realistically perhaps, by its linear or monotonic form, treating all phase changes as delays. In this connection all phase changes except one (that starting at Ct 2.9) were devoid of transients.

Earlier results with cockroaches (*Leucophaea maderae*) showed only small phase shifts even with light pulses of  $2,000 \text{ lx}$  (about  $800 \mu\text{W cm}^{-2}$ ) and 12 h duration<sup>14</sup>. But other results for the same species<sup>15</sup> have demonstrated strong type 0 resetting with very intense light ( $80,000 \text{ lx}$ , about  $32,000 \mu\text{W cm}^{-2}$ ) and about 12 h duration. The intensity of the light required to elicit a strong phase response curve in *L. maderae* is therefore about two orders of magnitude greater than in *N. cinerea*. Both of these studies, however, show that type 0 response curves can be obtained for 'behavioural rhythms' (presumably using compound eyes as photoreceptors, and having a neural output), provided that the energy of the perturbing signal is sufficiently high.

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- Pittendrigh, C. S. in *Circadian Clocks* (ed. Aschoff, J.) 277–297 (North-Holland, Amsterdam 1965).
- Aschoff, J. in *Circadian Clocks* (ed. Aschoff, J.) 95–111 (North-Holland, Amsterdam, 1965).
- Winfree, A. T. *J. theor. Biol.* **28**, 327–374 (1970).
- Saunders, D. S. *J. comp. Physiol.* **110**, 111–133 (1976).
- Minis, D. H. in *Circadian Clocks* (ed. Aschoff, J.) 333–343 (North-Holland, Amsterdam, 1965).
- De Coursey, P. J. *Cold Spring Harb. Symp. quant. Biol.* **25**, 49–55 (1960).
- Pittendrigh, C. S. & Daan, S. *J. comp. Physiol.* **106**, 223–355 (1976).
- Truman, J. W. & Riddiford, L. M. *Science* **167**, 1624–1626 (1970).
- Loher, W. J. *J. comp. Physiol.* **79**, 173–190 (1972).
- Nishitsutsuji-Uwo, J. & Pittendrigh, C. S. *Z. vergl. Physiol.* **58**, 1–46 (1968).

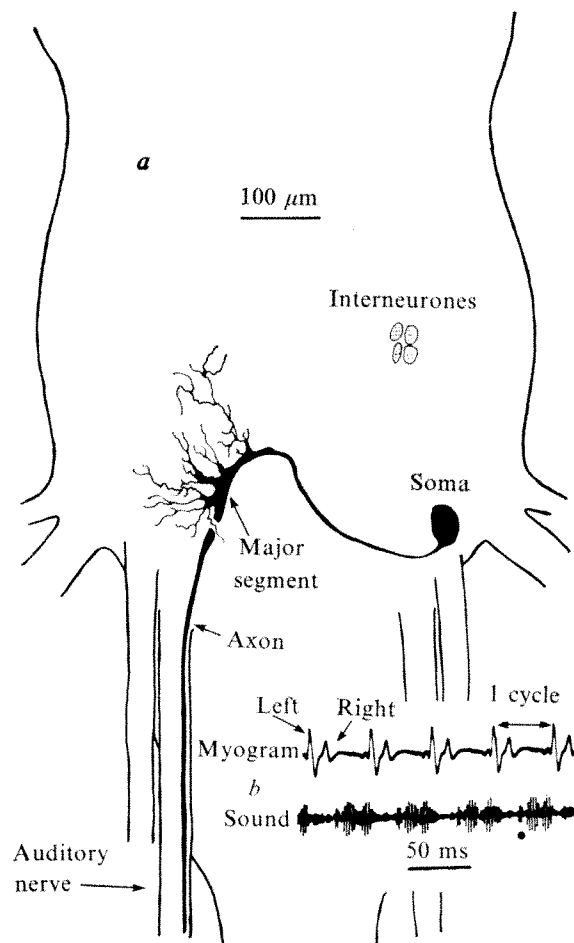
- Roberts, S. K. de F. *J. comp. Physiol.* **88**, 21–30 (1974).
- Brady, J. in *Biochronometry* (ed. Menaker, M.) 517–526 (National Academy of Sciences, Washington DC, 1971).
- Truman, J. W. in *Circadian Rhythmicity, Proc. int. Symp. Circ. Rhythmicity* 111–135 (Pudoc, 1971).
- Roberts, S. K. de F. *J. cell. Comp. Physiol.* **59**, 175–186 (1962).
- Wiedemann, G. *Z. Z. Naturforschung* (in the press).

## Neuronal generation of singing in a cicada

CICADA song may be regarded as a simple rhythmical behaviour because it is highly stereotyped and involves regular spikes in a single pair of motoneurons. It was the subject of an early study of intracellular activity in central neurones<sup>1</sup>. Here I show that the neuronal generator for song of the Australian bladder cicada, *Cystosoma saundersii* (Westw.) is continually active at twice the frequency of motoneurone spikes and, in common with other rhythmical behaviours in arthropods<sup>2–4</sup>, involves non-spiking interneurons.

The song of a cicada is composed of a regular series of sound pulses, each pulse being produced by a snap-like collapse of a stiff ribbed structure, a tymbal<sup>5–8</sup>. There is a tymbal on each side of the anterior of the abdomen. Collapse of a tymbal is caused by a twitch contraction of its tymbal muscle, and the inherent elasticity of the tymbal restores its original shape. Each tymbal muscle is in-

**Fig. 1 a**, Structure of a tymbal motoneurone, viewed dorsally. The abdominal and metathoracic ganglia of *C. saundersii* are fused, and their outline is drawn. The motoneurone was stained by back-filling with dilute (2%)  $\text{CoCl}_2$ . Somata of four interneurons, which were consistently stained by this method—probably trans-synaptically<sup>10</sup>—are also shown. Most of the processes of the motoneurone, as well as its soma, lie near the dorsal surface of the ganglion. **b**, Calling song of *C. saundersii*—sound produced, and simultaneous myogram recorded by a pair of silver electrodes implanted in the left tymbal muscle.



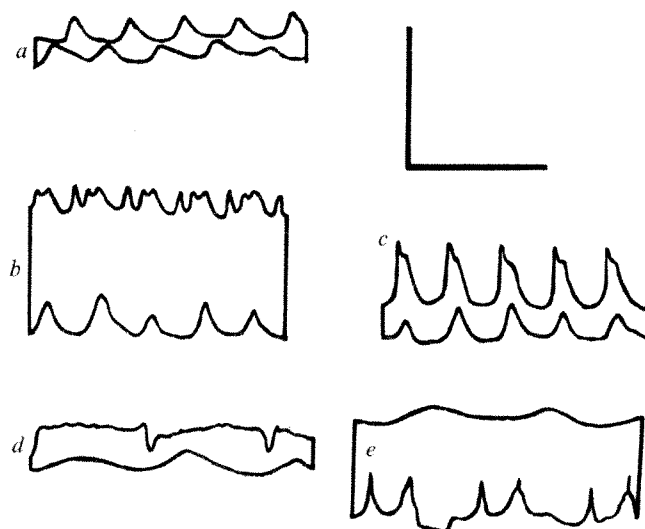
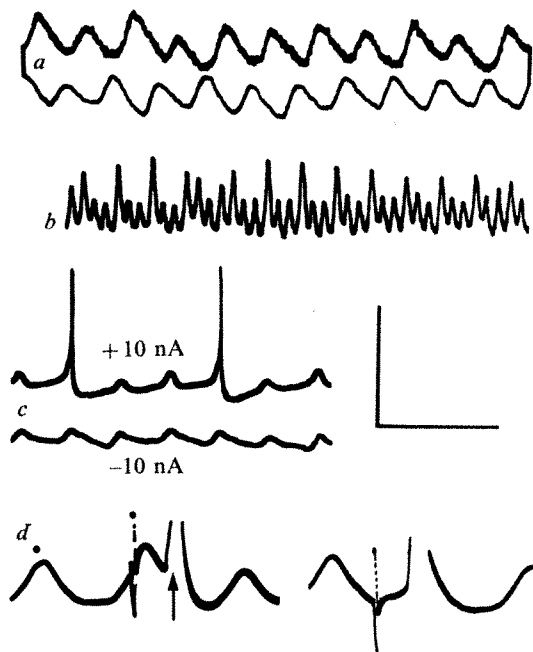


nervated by a single motoneurone, one spike in which is sufficient, in *C. saundersii*, to elicit a twitch contraction of the muscle (*C. saundersii* has neurogenic tymbal muscles; some species have myogenic tymbal muscles<sup>6-8</sup>). Figure 1a shows the geometry of a tymbal motoneurone. The pattern of spikes in tymbal motoneurons during the 'calling' song of *C. saundersii* is shown in Fig. 1b; it is a rather unusual rhythmical activity in that there is a delay of one quarter of a cycle between the spike in one tymbal motoneurone and the spike in its contralateral partner. Either the left or the right tymbal motoneurone may lead and, following short pauses when one or both motoneurons stop spiking, the motoneurons often swap the lead. 'Protest' songs, which a cicada produces when disturbed or when electrical shocks are applied to its brain, are similar although usually of lower spike frequency.

Intracellular recordings from tymbal motoneurons reveal regular, smooth waves in membrane potential (Fig. 2a, b). These waves occur continually, whether or not the cicada is singing. Waves in the left and right motoneurons have the same frequency, which suggests that they originate within a shared oscillator mechanism, but are half a cycle out of phase (Fig. 2a). In recordings from different animals at varying times of day the frequency of the waves ranges from 55 to 80 Hz, although in any particular recording session (up to 2 h) waves have a constant frequency, with only 1 in 200 waves being slightly longer or shorter than usual.

Two sets of observations show that the waves originate in interneurons rather than in the motoneurons them-

**Fig. 2** Intracellular recordings from tymbal motoneurons in a non-singing cicada. Recordings were made with strong glass electrodes filled with 2 M potassium acetate and with resistances 50–80 M $\Omega$ . The electrodes were driven through the intact sheath. In a, recordings are from somata; in other traces they are from unidentified processes. a, Waves recorded simultaneously from left (upper trace) and right (lower trace) tymbal motoneurons. b, Variation in wave amplitude immediately following a period of vigorous singing. c, Effects of passing current into a tymbal motoneurone. Current was passed through the recording electrode by means of a bridge circuit. c, Upper record, depolarising current elicits spikes on top of some waves. The spikes reached the tymbal muscle. Lower record, hyperpolarising current alters the shape but not the frequency of the waves. d, Antidromic spikes which follow, by 9 ms, shocks applied to the tymbal muscle, do not effect the timing or the amplitude of the next wave. Arrow denotes antidromic spike. Calibrations—a, 4 mV, 40 ms; b, 4 mV, 200 ms; c, 40 mV, 40 ms; d, 20 mV, 20 ms.



**Fig. 3** Recordings of phase-locked potentials in tymbal motoneurons and presumed interneurons of non-singing cicadas. a, Intracellular recordings from a tymbal motoneurone (upper trace) and from a non-spiking interneurone (lower trace). b, Intracellular recordings from a tymbal motoneurone (lower) and a non-spiking interneurone (upper). Wave frequency, 65 Hz. c, Hyperpolarising ( $-10$  nA) the interneurone of b alters its waveform and reduces the frequency of waves in it and in the motoneurone to 64.5 Hz. d, An interneurone (upper trace) which spiked once during each wave in a tymbal motoneurone (lower). The neuron is presumed to be an interneurone because no spikes in the motor nerves could be correlated with the frequency of waves in the tymbal motoneurons. e, An interneurone (lower) which spiked twice during each wave in a tymbal motoneurone (upper). Calibrations—a, motoneurone 10 mV, interneurone 1 mV, 40 ms; b, c, interneurone 2 mV, motoneurone 10 mV, 40 ms; d, interneurone 2 mV (a.c., extracellular), motoneurone 20 mV, 20 ms; e, motoneurone 5 mV, interneurone 1 mV, 20 ms.

selves. First, depolarising or hyperpolarising a motoneurone does not alter the frequency of the waves, but can affect their shape (Fig. 2c). Second, an antidromic spike in a tymbal motoneurone does not affect the timing or the amplitude of subsequent waves (Fig. 2d). The motoneurons do not seem to be directly coupled by an electrical or a chemical synapse because an antidromic or orthodromic spike in one tymbal motoneurone produces no recognisable effect on its partner. Each motoneurone receives inputs from different interneurons because there is no significant correlation between the amplitude of a wave in one motoneurone and the amplitude of the next wave in its partner. In the recording from which Fig. 2a is taken, for example, the correlation coefficient for amplitudes of sequential waves in the left and right tymbal motoneurons, calculated by a method of least squares, is 0.275, which is not significant.

Two observations strongly suggest that non-spiking interneurons drive the waves in the motoneurons. First, the waves are very smooth—discrete post-synaptic potentials have never been seen in them. Second, intracellular recordings have been made from a number of non-spiking interneurons with activity phase-locked to the waves in the motoneurons (Fig. 3a, b). The evidence that these neurons were non-spiking interneurons is that neither direct, strong depolarisation nor stimulation of motor axons elicited spikes in them. It is likely that these interneurons are involved in singing because the frequency of the waves in the tymbal motoneurons, which is twice that of spikes during song, is not related to that of any other rhythmical behaviour, such as ventilation, flight or walking. Unfortunately, passing current into an interneurone rarely affected the frequency of the waves, either in the interneurone or in a motoneurone. One exception is shown in Fig. 3b, c, where hyperpolarising the inter-

neurone caused a small but reversible decrease (65–64.5 Hz) in the frequency of waves in both the interneurone and the motoneurone and a change in the waveform in the interneurone. Possibly several interneurons are involved in generating the song rhythm and communicating it to the motoneurons, and passing current into only one interneurone has little effect on the rhythm generator. In addition to the non-spiking interneurons several interneurons which produce spikes phase-locked to the waves in the motoneurons were found (Fig. 3d, e).

When a *C. saundersii* sings each tymbal motoneurone produces a spike on every second wave (Fig. 4a). The frequency of tymbal motoneurone spikes and of sound pulses generated by each tymbal is therefore half that of the waves in the tymbal motoneurons. Because the waves in the left and right motoneurons are exactly out of phase, spikes in them are separated by one quarter of a cycle. Often, in 'protest' songs, spikes occur less regularly than every second potential (Fig. 4b, c)—the rule seems to be that spikes never occur on sequential waves.

The reason for this limit to spike frequency is unclear, but there are three observations which may indicate relevant factors. First, some waves which do not culminate in a spike reach more positive potentials than the take-off potential for spikes (Fig. 4c). Bearing in mind that recordings were probably never made from the spike initiation region of a tymbal motoneurone, the simplest explanation for this is that interneurons other than those

involved in producing the waves in a non-singing cicada are involved in producing spikes. Second, each of the largest waves is often followed by a much smaller one (for example, Fig. 2b). This is not due to direct negative feedback from a motoneurone because an antidromic spike does not affect the amplitude of subsequent waves and there is no correlation between the amplitude of one wave and the amplitude of the wave following it in the same motoneurone (range of serial correlation coefficients for 15 recordings: 0.04 to 0.43). Third, bursts of spikes in a tymbal motoneurone were never seen and even depolarising currents as great as 200 nA injected into a tymbal motoneurone soma produced only single spikes.

It may be a general feature of rhythmical behaviours that their neuronal rhythm generators are continually active. In quiescent locusts the neuronal oscillator for flight produces subthreshold depolarisations at flight frequency in motoneurons<sup>9</sup>. To use a mechanical analogy, nervous systems may well use 'clutches for engaging gear' rather than 'starter motors' in producing rhythmical movements.

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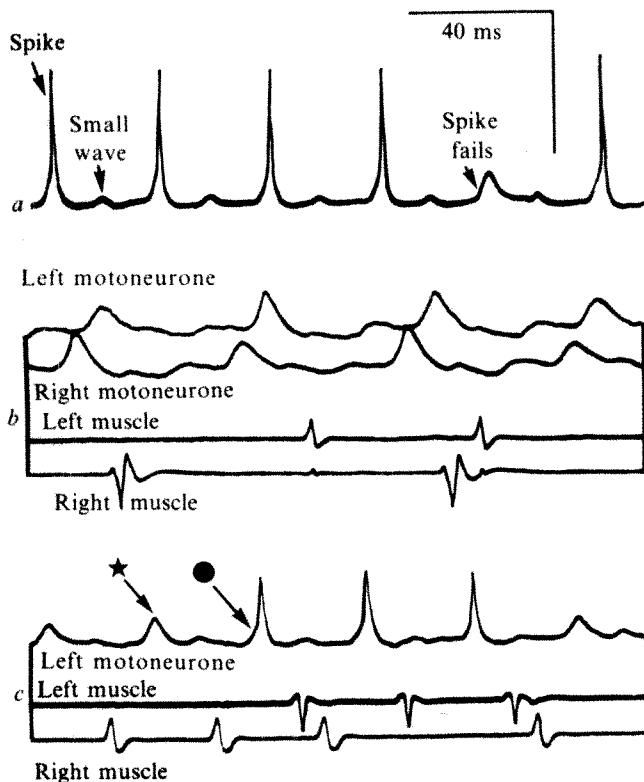
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- <sup>1</sup> Hagiwara, S. & Watanabe, A. *J. Cell. comp. Physiol.* 47, 415–428 (1956).
- <sup>2</sup> Mendelson, M. *Science* 171, 1170–1173 (1971).
- <sup>3</sup> Pearson, K. G. & Fournier, C. R. *J. Neurophysiol.* 38, 33–52 (1975).
- <sup>4</sup> Burrows, M. & Siegler, M. V. *S. Nature* 262, 222–224 (1976).
- <sup>5</sup> Reaumur, R. A. F. de *Mémoires pour Servir à l'Histoire des Insectes* 5, 145–206, Paris, (1740).
- <sup>6</sup> Pringle, J. W. S. *J. exp. Biol.* 31, 525–560 (1954).
- <sup>7</sup> Hagiwara, S. *Physiol. comp. Oecol.* 4, 142–153 (1955).
- <sup>8</sup> Young, D. J. *comp. Physiol.* 79, 343–362 (1972).
- <sup>9</sup> Burrows, M. *J. exp. Biol.* 63, 713–733 (1975).
- <sup>10</sup> Strausfeld, N. J. & Obermayer, M. *J. comp. Physiol.* 110A, 1–12 (1976).

**Fig. 4** Intracellular recordings from tymbal motoneurons when a *C. saundersii* is induced to sing by stimulating its brain electrically. *a*, Vigorous singing—spikes alternate with small waves. One large wave, where the spike fails, reaches a more positive potential than the take-off potential for spikes. *b*, Potentials recorded simultaneously from left and right somata. Spikes, identifiable by their sharp rising phase and from the muscle recordings, are rather infrequent, occurring at most on every third wave (left motoneurone). Note that not every large wave gives rise to a spike. *c*, The electrode for this recording had penetrated the ganglion near the origin of the auditory nerve (see Fig. 1). ★, Some waves reach a more positive potential than ●, the take-off potential for spikes. Calibrations—*a*, 40 mV, 40 ms; *b*, *c*, 20 mV, 40 ms.



## Site of *pcd* gene action and Purkinje cell mosaicism in cerebella of chimaeric mice

PURKINJE cell degeneration, *pcd*, is a new autosomal recessive mutation in mice. Affected animals are characterised by a moderate ataxia that results from the postnatal loss of virtually all cerebellar Purkinje cells<sup>1</sup>. We describe here experiments to determine whether the *pcd* gene acts intrinsically within the Purkinje cell or whether the degeneration is secondary to a primary lesion in some other cell type. In this study, experimental chimaeras were made which contained mixtures of mutant (*pcd/pcd*) and genotypically normal (+/+) cells (these chimaeras are denoted *pcd/pcd* ↔ +/+). The results indicate that the *pcd* gene does act within the Purkinje cell. The chimaeras have also been used to examine the surviving population of +/+ Purkinje cells for evidence of clonal development. Surprisingly, these preliminary studies reveal no evidence of clones; the distribution of cells derived from the +/+ component seems random.

The *pcd/pcd* ↔ +/+ chimaeras were produced by Tarkowski<sup>2</sup> and Mintz<sup>3</sup> techniques of aggregation of eight-cell embryos as described previously<sup>4</sup>. Since *pcd/pcd* males are sterile, embryos were obtained from *pcd/pcd* females that were mated to +/*pcd* males so only 50% of the chimaeras were expected to be homozygous for *pcd*. Five chimaeras were produced. One female chimaera was proven to be homozygous for *pcd* by progeny testing. In addition, two male chimaeras were also homozygous for *pcd* as evidenced by their abnormal sperm (a pleiotropic effect of the *pcd* mutation<sup>1</sup>) and loss of cerebellar Purkinje cells. All three *pcd/pcd* ↔ +/+ chimaeras appeared behaviourally normal. They were not, however, subjected to any special behavioural testing.

In all three chimaeras the cerebella were mosaics, for it was obvious in histological sections that many Purkinje cells had degenerated. Although the gene was expressed in the chimaeras,

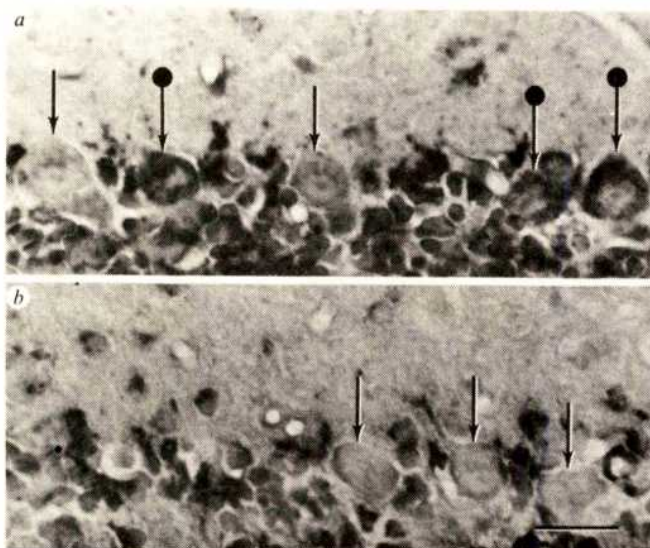


that is not evidence that it acts within the Purkinje cells. If the *pcd* locus exerts its primary effect within the Purkinje cell and is not cured by the presence of normal cells, then in the chimaera the cells that are *pcd/pcd* will degenerate and the surviving cells will be the  $+/+$  cells. Alternatively, if the degeneration of Purkinje cells is a secondary effect of the *pcd* locus acting in another cell type, then the surviving cells would be a mixture of *pcd/pcd* and  $+/+$  cells. To determine the genotypes of the surviving cells, it is necessary to use an independent cell marker.

One of the *pcd* chimaeras was also chimaeric for the structural gene for  $\beta$ -glucuronidase. This enzyme has been used previously as a cell marker in other tissues<sup>5,6</sup>. In the *pcd* chimaera, the *pcd/pcd* cells were homozygous for *Gus<sup>b</sup>*, the high enzyme activity allele, whereas the  $+/+$  cells were homozygous for *Gus<sup>h</sup>*, the low activity allele. This chimaera is denoted *pcd/pcd Gus<sup>b</sup>/Gus<sup>b</sup> ↔  $+/+$  Gus<sup>h</sup>/Gus<sup>h</sup>*. A new, more sensitive histochemical procedure, developed by Feder<sup>6</sup> was used. This procedure is limited in its use as a central nervous system (CNS) cell marker, but it can be used to determine the genotype of Purkinje cells (R. J. M. and R. L. Sidman, unpublished). In *Gus<sup>b</sup>/Gus<sup>b</sup>* non-chimaeric controls, all of the Purkinje cells show enzyme activity as evidenced by the red reaction product. In *Gus<sup>h</sup>/Gus<sup>h</sup>* non-chimaeric controls, none of the Purkinje cells has sufficient enzyme activity to produce any noticeable staining. In *Gus<sup>b</sup>/Gus<sup>b</sup> ↔ Gus<sup>h</sup>/Gus<sup>h</sup>* chimaeras (both components being  $+/+$  at the *pcd* locus), a mosaic of stained and unstained Purkinje cells is observed (Fig. 1a). Virtually all Purkinje cells can be assigned to one class or the other. When sections from the *pcd/pcd Gus<sup>b</sup>/Gus<sup>b</sup> ↔  $+/+$  Gus<sup>h</sup>/Gus<sup>h</sup>* chimaera were stained by this technique, none of the surviving Purkinje cells showed enzyme activity (Fig. 1b), indicating that they were *Gus<sup>h</sup>/Gus<sup>h</sup>* and therefore  $+/+$  at the *pcd* locus. Thus, in the chimaera, the *pcd/pcd* Purkinje cells have degenerated leaving the genetically normal  $+/+$  cells.

The simplest interpretation of this is that the genic alteration

**Fig. 1** Sagittal sections (7  $\mu$ m) of cerebellar cortex stained histochemically for  $\beta$ -glucuronidase and counterstained with methyl green. The mice were perfused with cold (0–4 °C) 4% formaldehyde in phosphate buffer. The brains were processed and stained as described by Feder<sup>6</sup>. The sections were stained for 3 d at 37 °C with fresh substrate added daily. In these photomicrographs the red precipitate at the sites of glucuronidase activity seems dark gray. *a*, Cerebellar cortex of a *Gus<sup>b</sup>/Gus<sup>b</sup> ↔ Gus<sup>h</sup>/Gus<sup>h</sup>* chimaera. Some of the large Purkinje cells are stained (arrows with dots) indicating they are from the *Gus<sup>b</sup>* component; others are not stained (arrows) and are, therefore, *Gus<sup>h</sup>*. *b*, Cortex of a *pcd/pcd Gus<sup>b</sup>/Gus<sup>b</sup> ↔  $+/+$  Gus<sup>h</sup>/Gus<sup>h</sup>* chimaera. None of the surviving Purkinje cells is stained (arrows) indicating they are from the  $+/+$  *Gus<sup>h</sup>/Gus<sup>h</sup>* component. To the left is a gap left by degenerated Purkinje cells. In both *a* and *b* the other intense staining in the Purkinje cell layer is probably in Golgi epithelial cells (Bergmann glia). Scale bar, 20  $\mu$ m.



**Fig. 2** Distribution of surviving  $+/+$  Purkinje cells in a *pcd/pcd ↔  $+/+$*  chimaera in which 75% of the cells have degenerated. With a drawing tube, the positions of Purkinje cells along the Purkinje cell layer of the lobulus centralis in 20- $\mu$ m sagittal sections were traced on tracing paper. The presence of nuclei and nucleoli were also marked to help eliminate cells present in adjacent sections. After aligning the tracings by superimposition, a map reader was used to measure distances along the Purkinje cell lamina. The positions of the cells were then plotted in straight lines on graph paper. Twenty-five serial sections (500  $\mu$ m) were recorded. The size of the dots is scaled to the average Purkinje cell diameter. Thus, the figure represents the location of cells as they would appear if the folium was flattened out and viewed from above. A two-dimensional reconstruction such as this might change the shape of a patch or clone of cells, but it would not disrupt it. Throughout the rest of the cerebellum of this chimaera, the proportion of cells that survived was relatively constant; there were no regions where the density of Purkinje cells was normal and no regions where they had all degenerated. Thus, the portion reconstructed is not at an interface between large patches of mutant and normal cells. Scale bar, 100  $\mu$ m.



at the *pcd* locus is affecting the Purkinje cell directly. This conclusion is supported by an ultrastructural study of *pcd/pcd* Purkinje cells in which a unique series of abnormalities in Purkinje cells was observed. Nothing was observed in this earlier study that would suggest the gene was acting anywhere else in the cerebellum other than the Purkinje cell<sup>7</sup>. One alternative is that the *pcd* locus is acting in some other cell, also of the *pcd/pcd* genotype, with which the Purkinje cell must interact. This seems highly unlikely, for at least some *pcd/pcd* Purkinje cells should have been close enough to  $+/+$  cells of this 'other' cell type during development to be saved, but none was.

Since the surviving Purkinje cells in the chimaera are all of the same genotype, they had, at some time in development, common ancestors. Their distribution can be studied for evidence of clones that might be of developmental, anatomical and/or functional significance. In single sections the mosaicism is apparently extremely fine with some 'patches' being a single cell. This, however, must be cautiously interpreted, as what seems to be a single, isolated cell could be on the edge of a much larger patch or there could be narrow clones running perpendicular to the plane of section. The Purkinje cell layer is relatively well-suited for serial reconstruction for it is a single cell-thick lamina so the positions of cells can be plotted in two dimensions. A serial reconstruction showing the distribution of surviving Purkinje cells in a portion of the central lobule (lobulus centralis<sup>8</sup>) of one of the *pcd* chimaeras is shown in Fig. 2. There does not seem to be any pattern or evidence of clones; the distribution seems to be random.

Although it might seem unlikely, the random distribution could be the result of rearrangement of the  $+/+$  cells after the *pcd/pcd* cells degenerate. This possibility was investigated by examining chimaeras in which there was no cell loss but in which the genotype of Purkinje cells was determined with the  $\beta$ -glucuronidase cell marking technique. Sections from a *Gus<sup>b</sup>/Gus<sup>b</sup> ↔ Gus<sup>h</sup>/Gus<sup>h</sup>* chimaera were analysed to estimate the number of cells in a clone of Purkinje cells. The estimate is based on a comparison of the mean patch size (contiguous



**Table 1** Mean patch size and estimate of clone size for *Gus<sup>b</sup>/Gus<sup>b</sup>* Purkinje cells in a *Gus<sup>b</sup>/Gus<sup>b</sup> ↔ Gus<sup>b</sup>/Gus<sup>h</sup>* chimera.

Mean no. of <i>Gus<sup>b</sup>/Gus<sup>b</sup></i> cells per patch				
1.245 ± 0.071	1.367 ± 0.100	1.196 ± 0.051	1.298 ± 0.091	1.217 ± 0.063
Cells				
Observed mean patch size (total)	1.259 ± 0.033			
Expected in random linear array	1.212			
Clone (observed/expected)	1.030 ± 0.021			

Each of the five means is based on a complete sagittal section through the vermis of a chimera in which 17.5% of the cells were *Gus<sup>b</sup>/Gus<sup>b</sup>* (that is, stained). A total of 1,947 cells were scored. There were 270 patches (that is, groups of contiguous cells of like genotype). The mean patch size expected in a random linear array is estimated by  $1/(1-p)$  where  $p$  is the proportion of cells of that genotype in the population ( $p_{Gus^b} = 0.175$ ). For an approximation of the number of cells per patch in a two-dimensional array, the numbers could be squared. That would not, however, alter the conclusion that the distribution is near random.

cells of like genotype) with the mean expected in a random array<sup>9</sup>. In a purely random array, there would be one cell per clone. The data, shown in Table 1, yield an estimate of 1.03 Purkinje cells per clone. Thus, even in chimaeras in which there is no cell loss, the Purkinje cells seem to be almost randomly distributed. A fine-grained mosaicism has also been observed by Dewey *et al.*<sup>10</sup> using other cell marking techniques.

These studies indicate that in the cerebellum the *pcd* locus exerts its primary effect within the Purkinje cell. The *pcd* locus is pleiotropic; its other effects include loss of retinal photoreceptor cells<sup>11</sup>, mitral cells in the olfactory bulbs<sup>1</sup> and males have abnormal (or degenerating) sperm<sup>1</sup>. These other effects were expressed in the chimaeras, but it could not be determined whether the *pcd* locus was also acting directly within those cell types.

It is well established that most cortical neurones, including Purkinje cells, are born in germinal centres such as the embryonic ventricular zone and then migrate to the position they will occupy in the adult cortex<sup>12</sup>. Although they do migrate, the migration seems to be radial and one might expect that daughter cells would remain in proximity to one another which would give rise to clones of cells of the same genotype in adults. In this study, and in others<sup>10,13</sup>, however, there is little evidence of clones which suggests that at some time in development there must be a considerable amount of cell mixing. Additional studies are obviously needed to confirm this finding and to determine when the mixing occurs. It will also be of interest to examine other cell types for Purkinje cells are unusual in that they are spread out into a single cell lamina during the massive growth and foliation of the cerebellum.

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1. Mullen, R. J., Eicher, E. M. & Sidman, R. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 208–212 (1976).
2. Tarkowski, A. K. *Nature* **190**, 857–860 (1961).
3. Mintz, B. *Am. Zool.* **2**, 432 (1962).
4. Mullen, R. J. & Whitten, W. K. *J. exp. Zool.* **178**, 165–176 (1971).
5. Condamine, H., Custer, R. P., & Mintz, B. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2032–2036 (1971).
6. Feder, N. *Nature* **263**, 67–69 (1976).
7. Landis, S. C. & Mullen, R. J. *J. comp. Neur.* (in the press).
8. Sidman, R. L., Angevine, J. B., Jr & Taber Pierce, E. *Atlas of the Mouse Brain and Spinal Cord* (Harvard University Press, Cambridge, Massachusetts 1971).
9. West, J. D. *J. Theor. Biol.* **50**, 153–160 (1975); *J. Embryol. exp. Morph.* **35**, 445–461 (1976).
10. Dewey, M. J., Gervais, A. G. & Mintz, B. *Dev. Biol.* **50**, 68–81 (1976).
11. Mullen, R. J. & LaVail, M. M. *Nature* **258**, 528–530 (1975).
12. Sidman, R. L. & Rakic, P. *Brain Res.* **62**, 1–35 (1973).
13. Mullen, R. J. in *The Clonal Basis of Development* (36th Symp. Soc. Dev. Biol. (Academic, New York, in the press)).

## Selective expression of xenotropic virus in congenic HRS/J (hairless) mice

GENETIC factors strongly influence susceptibility to leukaemia in mice. In some instances leukaemogenesis hinges on only a few genes, or even a single gene<sup>1</sup>. The hairless mutation is an example of this. Homozygotes for this autosomal recessive gene (*hr/hr*) have a high incidence of thymic leukaemia (45% at 8–10 months), whereas the incidence in heterozygotes (*hr/+*) is low (1% at 8–10 months)<sup>2</sup>. The leukaemogenic effect produced by homozygosity for *hr* is unknown. Here we report that thymuses of *hr/hr* mice produce high titres of xenotropic viruses, whereas thymuses of *hr/+* mice do not. Furthermore, viruses with a broadened host range were isolated from thymuses of preleukaemic *hr/hr* mice, but not from any tissue of *hr/+* mice. We propose that the mutant gene *hr* specifies circumstances that augment production of xenotropic virus by the thymus. This phenomenon may be an important step in the development of thymic leukaemia.

The virology of *hr/hr* and *hr/+* mice has not been extensively studied. Using a complement fixation test, Meier's group found that adult and embryo tissues of both homozygotes and heterozygotes contained similar amounts of murine leukaemia virus antigen<sup>3</sup>. But, using an XC plaque assay to test tail extracts the same authors later reported that the *hr/hr* mouse expressed several times higher amounts of ecotropic Type C virus than the *hr/+* animal<sup>3</sup>.

In a preliminary experiment we found that the ecotropic virus in HRS/J mice, the strain that carries the *hr* gene, was N-tropic. Therefore, we measured ecotropic virus in a modified XC plaque assay using NIH-3T3 cells<sup>4</sup>. As shown in Fig. 1a, N-tropic virus was readily detected in spleen, thymus, and bone marrow of both *hr/hr* and *hr/+* mice. Titres of virus increased with age, most notably in the thymus. But, at no time was there a difference in titre of infectious ecotropic virus between the two types of mice. It is therefore unlikely that the difference in the incidence of leukaemia can be explained by a difference in the expression of ecotropic virus.

Xenotropic virus was assayed by a focus-induction assay in Moloney sarcoma virus-transformed, non-producer cat cells<sup>5</sup> and a fluorescent antibody focus method involving mink lung cells<sup>6</sup>. With the cat cell assay, we detected small amounts of xenotropic virus in thymuses of 2-month-old mice, but there was no difference between *hr/hr* and *hr/+* mice at this age (Fig. 1b). The titre of xenotropic virus in thymuses of 8-month-old *hr/hr* mice increased remarkably, however, whereas virtually no increase was found in *hr/+* mice. Low titres of xenotropic virus were also found in spleen and bone marrow of some *hr/hr* and *hr/+* mice. Therefore, increased expression of xenotropic virus was specifically related to the age, tissue and genotype of the mouse.

We confirmed this observation with the mink cell assay (Fig. 1c): at 8 months of age the expression of xenotropic virus in thymuses of *hr/hr* mice was, on the average, 100 times more than that in thymuses of *hr/+* mice. In other tissue, low titres of xenotropic virus were occasionally detected. We failed to find any clear-cut morphological changes in the infected mink cells.

We next characterised further the xenotropic virus that infected the mink cells. After several *in vitro* passages of the infected mink cells, the supernatant was filtered (Millipore, 45 µm) and used to infect fresh mink cells. After three or four more passages the culture supernatant was filtered and tested for infectivity on both mink and NIH-3T3 cells by means of the XC-plaque assay and the fluorescent antibody focus method. None of 48 isolates produced XC plaques on either NIH-3T3 or mink cells; 46/48 isolates

were pure xenotropic virus in that they failed to infect mouse cells, but readily infected mink cells (Table 1). But, 2/7 isolates from thymuses of 8-month-old *hr/hr* mice infected both mouse and mink cells. Although we have not yet cloned the latter isolates, it is unlikely that they are

simple mixtures of ecotropic and xenotropic viruses because: (1) they were infectious in mouse cells after having been passed more than 10 times in mink cells, and (2) they were totally negative in the XC test. We refer to these viruses as polytropic because of their broad host range and to

**Fig. 1** Expression of endogenous viruses in HRS/J mice. Ecotropic and xenotropic viruses were quantitated by infectious centre assays; titres were expressed as  $\log_{10}$  of the number of plaques or foci per  $10^7$  cells used in the assay. Black columns represent the virus titres of *hr/hr* mice and open columns those of *hr/+* mice. *a*, Ecotropic virus. In this assay<sup>4</sup>,  $2 \times 10^5$  NIH-3T3 cells were plated in a 60-mm plastic Petri dish; 24 h later, the monolayer was treated with 2 ml of  $25 \mu\text{g ml}^{-1}$  DEAE-dextran for 60 min at  $37^\circ\text{C}$ , rinsed, and infected with serial dilutions of washed lymphoid cells. After 5 d the infected indicator cells were exposed to ultraviolet light and overlaid with  $1 \times 10^6$  XC cells. Two days later, the culture was methanol-fixed and Giemsa-stained and the number of plaques was counted. The medium was  $4 \times$  Eagle's minimum essential medium with 10% unheated foetal calf serum. *b*, Xenotropic virus detected by the cat cell assay<sup>6</sup>. The indicator cat cell (8C) monolayer was prepared as above, treated with DEAE-dextran for 30 min, rinsed and infected. Transformed cell foci produced by rescued MSV pseudotypes were counted 12 days after infection. The medium was McCoy's 5A medium with 15% heat-inactivated foetal calf serum. *c*, Xenotropic virus by the mink cell assay<sup>6</sup>. Preparation of the indicator cell monolayer and method of infection was the same as in *b*. Cultures were made in duplicate; one with  $20 \times 20$  mm coverslip and another without. On the fifth day, cells on the coverslip were acetone-fixed and stained with fluorescein isothiocyanate conjugated goat antiserum against Moloney virus (lot 5010101, NCI). Cultures without coverslips were maintained for 7–10 d to study possible morphological changes in infected mink cells and for subsequent isolation of the virus. The medium was McCoy's 5A medium with 10% unheated foetal calf serum. In all the infectious centre assays *in vitro*, medium was changed at 2–4 d intervals.

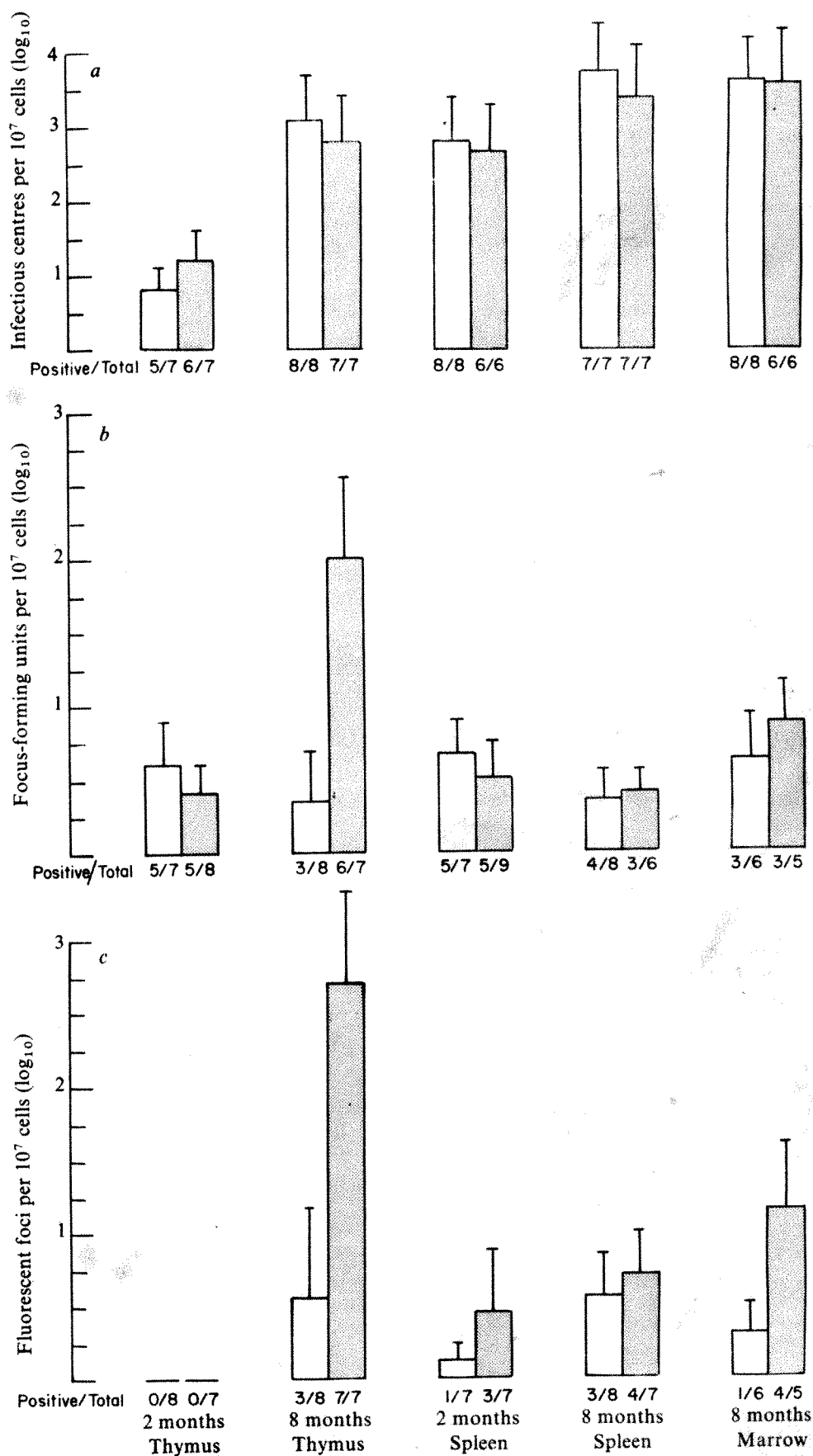


Table 1 Isolation of polytropic virus

Genotype	Age (months)	Polytropic/xenotropic		
		Thymus	Spleen	Bone marrow
<i>hr/hr</i>	2	0/2	0/3	0/3
	8	2/7	0/4	0/1
	Leukaemic	0/5	0/2	
<i>hr/+</i>	2	0/2	0/3	0/4
	8	0/3	0/1	0/3
	Leukaemic	0/2	0/3	

distinguish them from the amphotropic viruses of wild mice<sup>7</sup>. In a preliminary experiment, both an ecotropic and a xenotropic virus interfered with the infectivity of the polytropic virus for mouse and mink cells, respectively (data not shown). The polytropic isolates produced subtle morphological changes in infected mink cells, such as piling-up of cells or tiny cytoplasmic vacuolations, but these changes were not as clear as those reported by Hartley *et al.*<sup>8</sup> with the analogous AKR virus. Results of further characterisation of the polytropic isolates will be reported elsewhere. Several xenotropic isolates obtained from leukaemic tissue were also studied; none of the 12 samples was polytropic.

Titres of ecotropic and xenotropic viruses in leukaemic tissues of *hr/hr* and *hr/+* mice are shown in Fig. 2. There were no significant differences in titres of ecotropic virus between preleukaemic and leukaemic thymic tissues. By contrast, leukaemic thymic tissue had much lower titres of xenotropic virus than preleukaemic thymus tissue. These findings may be explained either by regulation of expression of the virus at different stages of leukaemogenesis or by a shift in cell types in the thymus.

Our results are comparable to those of Hartley *et al.*<sup>8</sup> in the AKR mouse. These workers demonstrated an increased

expression of xenotropic virus in thymuses of preleukaemic AKR mice<sup>8</sup>. Some isolates of this virus were polytropic and probably represent recombinant viruses<sup>8</sup>. The polytropic virus obtained from AKR mice produced no plaques in the XC test but productively infected mouse cells, as determined by an immunofluorescent method. The virus we obtained from hairless mice had similar properties. There are other similarities between the AKR and hairless systems: titres of ecotropic virus are high in all three types of mice; in contrast, expression of xenotropic and polytropic viruses is observed specifically in thymuses of AKR and *hr/hr* mice, but not in the *hr/+* mouse.

We stress here the outstanding advantage of the hairless mouse system: it is congenic, thus providing internal controls for analyses of the effects of one or at most very few genes in viral leukaemogenesis. Our results suggest that one effect of the mutant gene is to augment the production of xenotropic virus by the thymus. Genetic recombination between this agent and an ecotropic virus may give rise to the polytropic virus we detected. Experiments to test the oncogenicity of the latter agent are in progress.

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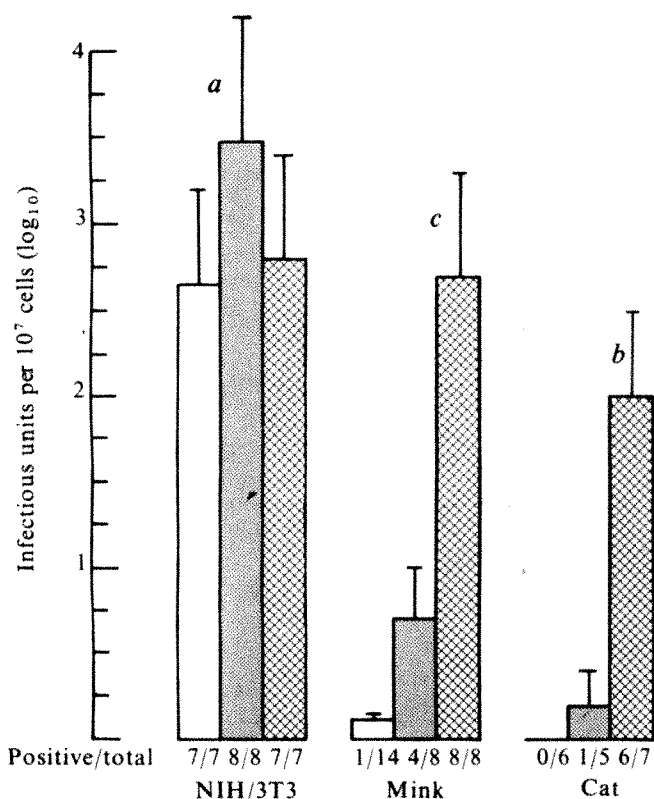
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1. Lilly, F., Pincus, T. *Adv. Cancer Res.* **17**, 231-277 (1973).
2. Meier, H., Myers, D. D. & Huebner, R. J. *Proc. natn. Acad. Sci. U.S.A.* **63**, 759-766 (1969).
3. Heiniger, H. J., Huebner, R. J. & Meier, H. J. *natn. Cancer Inst.* **56**, 1073-1074 (1976).
4. Melief, C. J. M. *et al. Proc. Soc. exp. Biol. Med.* **149**, 1015-1018 (1975).
5. Fishinger, P. J., Blevins, C. S. & Nomura, S. J. *Virology* **14**, 177-179 (1974).
6. Kawashima, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4680-4684 (1976).
7. Hartley, J. W. & Rowe, W. P. *J. Virol.* **19**, 19-25 (1976).
8. Hartley, J. W., Wolford, N. K., Old, L. J. & Rowe, W. P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 789-792 (1977).

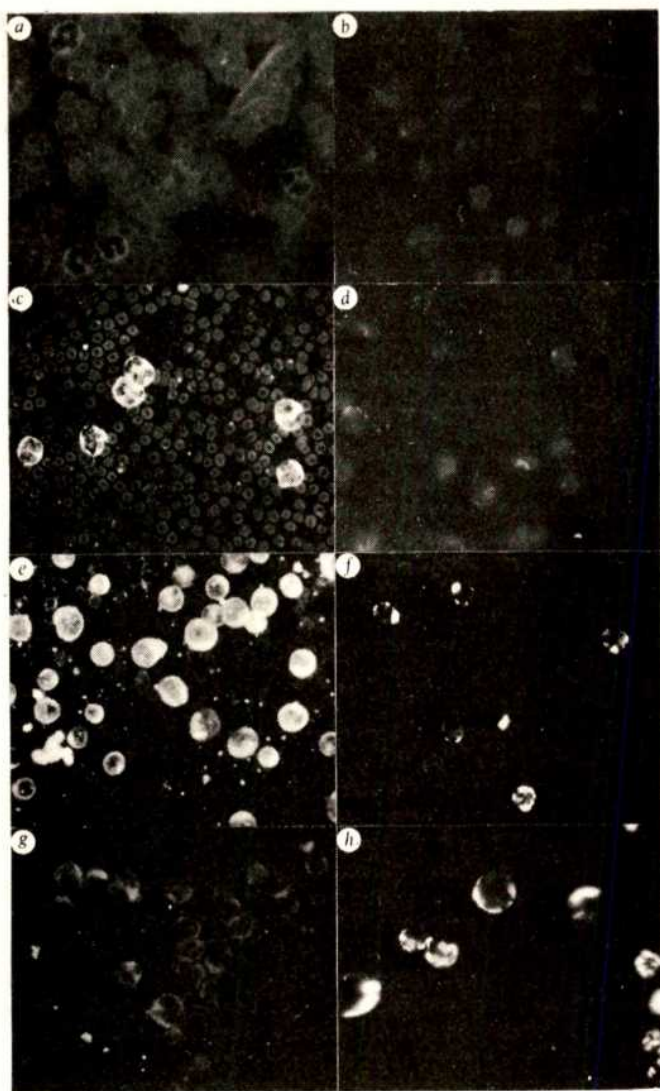
Fig. 2 Ecotropic and xenotropic viruses in preleukaemic and leukaemic thymus. *a*, Ecotropic virus; *b*, xenotropic virus (cat cell assay), *c*, xenotropic virus (mink cell assay). Black column represents the virus titre in leukaemic tissue of *hr/hr*; open column, *hr/+* leukaemic tissue; and hatched column, the titre in preleukaemic *hr/hr* thymus.



## A feline leukaemia virus- and sarcoma virus-induced tumour-specific antigen

BIOCHEMICAL and immunological methods of distinguishing between normal and malignant cells have had limited success. When studying cellular transformation, it is advantageous to study tumour viruses since they contain a definable quantity of genetic information for viral replication and for cell transformation. Viral gene products, such as viral structural antigens, can be studied in both normal infected and virus-transformed cells. Oncornavirus antigens are found in the cytoplasm and cell membrane of both normal and transformed infected cells<sup>1,2</sup>. These antigens are glycoproteins of the viral envelope or are internal viral proteins<sup>3-5</sup>. Non-viral tumour-specific antigens resulting from transformation of the host cell by the causative virus, are found in the tumour cell membrane of some tumours induced experimentally by polyoma<sup>6</sup> and SV40<sup>7</sup> DNA viruses; and in cells transformed by an oncornavirus, the Rous sarcoma virus<sup>8,9</sup>, but have not yet been found in any oncornavirus-induced leukaemic cell. Lymphosarcoma (LSA) or leukaemia of pet cats is caused by a contagiously spread oncornavirus, the feline leukaemia virus (FeLV)<sup>10</sup>. Antibody to the feline oncornavirus-associated cell membrane antigen (FOCMA)<sup>11,12</sup> is associated with resistance to LSA development in FeLV-infected pet cats<sup>13,14</sup> and with regression of feline sarcoma virus (FeSV) experimentally-induced fibrosarcomas<sup>15</sup>. To





**Fig. 1** Viable cell IFA tests for FOCMA and fixed cell IFA tests for intracytoplasmic FeLV antigens of cat cells. Normal feline leukocytes: *a*, FeLV antigen negative; *b*, FOCMA negative. Normal feline leukocytes: *c*, FeLV antigen positive; *d*, FOCMA negative. Lymphosarcoma cells: *e*, FeLV antigen positive; *f*, FOCMA positive. Lymphosarcoma cells: *g*, FeLV antigen negative; *h*, FOCMA positive. In the direct FOCMA test a 0.05ml aliquot of a  $15 \times 10^6$  ml<sup>-1</sup> washed cell suspension was incubated at 4 °C for 30 min with 0.05 ml 1:4 dilution of FeLV-infected cat serum which had a high titre (1:32) of antibody to FOCMA, but which had no FeLV-neutralising antibody. As a control, another 0.05ml aliquot of a washed cell suspension was incubated, in the same conditions, with 0.05 ml cat serum containing no FOCMA antibodies. After incubation, the cells were washed twice, mixed with the fluorescein isothiocyanate-conjugated rabbit anti-cat serum globulin (Sylvania, Millburn, New Jersey), diluted 1:10, and allowed to react on ice for 30 min. Cells were then washed twice and resuspended in 0.05 ml 50% glycerol in PBS. A minimum of 100 cells were counted from each cell preparation. When 50% of the cells had punctate membrane fluorescence, and when the number of cells fluorescing in the FOCMA test was at least 25% greater than the number of fluorescing B cells from the same preparation, the cat was considered to have FOCMA-positive lymphoid cells (that is, the cells were lymphoid tumour cells). In the FOCMA absorption test, an aliquot of the cat anti-FOCMA serum was used at two double dilutions below the 50% fluorescent endpoint. The diluted serum was then absorbed twice for 30 min each at 4 and 37 °C with an equal volume (a minimum of 0.2 ml) of washed packed test cells. The residual FOCMA antibody titre of the absorbed cat anti-FOCMA serum was then determined in a direct FOCMA test as described previously, and compared with that of unabsorbed (control) serum from the same aliquot. A 25% or greater reduction in the FOCMA antibody titre of the absorbed serum was considered a positive absorption, indicating that FOCMA was present on the B lymphoid cells and that these cells were tumour cells.

determine whether FOCMA is a tumour-specific antigen, we tested normal, LSA and fibrosarcoma cat cells for both FeLV antigens and FOCMA. We report here that FOCMA is a tumour-specific antigen induced by naturally occurring feline leukaemia and sarcoma viruses. Our observation, which was made in a random bred species, the pet cat, may have particular relevance for the diagnosis and therapy of naturally occurring lymphoid tumours of other animals, including man.

Cells from both FeLV-infected and uninfected healthy pet cats, and FeLV-infected and uninfected cats with naturally occurring LSA, were studied. All cells were tested for cytoplasmic FeLV structural antigens by the fixed cell immunofluorescent antibody (IFA) test<sup>16</sup>. Detection of FeLV antigens in the cytoplasm indicates that FeLV is replicating in these cells and that FeLV gp70 and p30 are present in their cell membranes<sup>4,5,16</sup>.

To determine whether FOCMA is expressed on lymphoid cells regardless of their cellular origin, tests to determine whether the cells were of T or B cell origin were also carried out. Lymphocytes were tested for their ability to form rosettes with guinea pig erythrocytes to determine whether they were of T cell origin, and for the presence of cell surface immunoglobulins (Ig) to determine whether they were of B cell origin<sup>17</sup>.

Two tests for FOCMA were carried out, the direct test and the absorption test using a standard reference serum (see Fig. 1)<sup>11,12</sup>. This reference serum was obtained from an FeLV-infected cat and contained FOCMA antibody at a titre of 1:32 but did not contain FeLV-neutralising antibody. The FOCMA reactivity of the serum could not be absorbed out with intact or disrupted FeLV, with purified FeLV gp70 or p30<sup>18,19</sup>, with foetal cat cells, or with normal cat lymphoid cells. FOCMA antibody was absorbed only by FeLV-producing or non-producing feline LSA cells. If the lymphoid cells were found to be of B cell origin they were tested for FOCMA by the absorption test. This was necessary since the fluorescein-conjugated rabbit anti-cat serum globulin used in the direct FOCMA test reacts with Ig-producing B cells and thus makes interpretation of the direct test impossible.

A total of 36 cats with histological diagnoses of LSA were studied. Twenty-seven of the cats had FeLV-producing LSA cells, whereas the LSA cells of the other nine cats were not replicating FeLV. Twenty-three out of the 36 LSAs (64%) were of T cell origin (Table 1). All 12 thymic LSAs and 10 of the 18 multicentric LSAs were T cell tumours. Both T and B cell markers were present in seven of the LSAs and were classified as mixed cell tumours. Only four LSAs were of B cell origin and three of these were LSAs of the gastrointestinal tract. No lymphocyte cell surface markers could be detected in two LSAs and these were classified as null cell tumours.

All 33 FeLV-producing LSA cell preparations obtained from 27 FeLV-infected LSA cats were positive for FOCMA (Table 2). Similarly, all 13 LSA cell preparations, which were not replicating FeLV, obtained from nine FeLV IFA test-negative LSA cats were also FOCMA positive. FOCMA was expressed on lymphoid tumour cells (Fig. 1) regardless

**Table 1** T and B cell origin and forms of feline lymphosarcoma

Lymphosarcoma form	No. of cats tested	T	Cellular origin B	Mixed	Null
Multicentric	18	10	1	5	2
Thymic	12	12	0	0	0
Alimentary	5	1	3*	1†	0
Unclassified	1	0	0	1	0
Totals	36	23	4	7	2

\* Two gastric LSA.

† Intestinal LSA.



**Table 2** Occurrence of FOCMA in cats with lymphosarcoma

Number of cats with lymphosarcoma	FeLV status of cats with lymphosarcoma	Tissues tested	Number tested	FOCMA	
				positive	negative
27	+	Lymphosarcoma cells obtained from:			
		Blood	15	15	0
		Thymus	9	9	0
		Lymph Nodes	6	6	0
		Stomach	1	1	0
		Spleen	1	1	0
		Kidney	1	1	0
		Total	33	33	0
		Normal blood lymphocytes from +cats with LSA	4	0	4
		Lymphosarcoma cells obtained from:			
9	—	Lymph nodes	4	4	0
		Thymus	3	3	0
		Stomach or intestine	2	2	0
		Blood	2	2	0
		Omentum	1	1	0
		Spleen	1	1	0
		Total	13	13	0
		Normal blood lymphocytes from —cats with LSA	4	0	4

of their FeLV status and T, B, mixed or null cell origin. By contrast, four out of four FeLV-infected and four out of four FeLV-negative morphologically normal lymphocyte preparations, which were obtained from the peripheral blood of cats with LSA who had no evidence of circulating leukaemic cells, were negative for FOCMA. A total of 63 normal cell preparations, 20 FeLV-infected and 43 FeLV uninfected, obtained from healthy cats were negative for FOCMA. Normal lymphocytes from 11 FeLV-infected and 22 uninfected cats were FOCMA negative. Infected neutrophils from six healthy cats and infected thymocytes from three foetuses were FOCMA negative. Uninfected neutrophils (eight cats), thymocytes (five cats), splenocytes (five cats) and myelocytes (three cats) were also negative for FOCMA.

Normal FeLV-infected and uninfected feline embryo lung fibroblast cell cultures were negative for FOCMA. Similarly, three non-lymphoid feline tumour cell cultures (one mammary adenocarcinoma, one osteosarcoma, and one anaplastic sarcoma) were also negative for FOCMA. Two fibrosarcoma cultures obtained from young cats with naturally occurring multicentric FeSV fibrosarcomas and granulocytic tumour cells from a cat with myelogenous leukaemia were, however, FOCMA positive, as was a dog fibrosarcoma culture from an FeSV-induced fibrosarcoma in a beagle puppy.

Our findings indicate that FOCMA is an FeLV- or FeSV-induced tumour-specific antigen expressed on the membranes of naturally occurring feline LSA, myelogenous leukaemia and multicentric fibrosarcoma cells. The finding that myelogenous leukaemia cells are FOCMA positive is important, since it indicates that FeLV can transform granulocytes in addition to lymphocytes. Since FOCMA has been induced by FeSV in cells of other species<sup>20</sup>, it is unlikely that FOCMA is an expression of an activated feline cellular gene. The expression of FOCMA on the membranes of FeLV non-producer feline LSA cells enables these cells to be used to study a defective leukaemia virus of a naturally occurring LSA. Such cells will be valuable

for comparative studies of lymphoid tumours of all animals, including man.

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- Klein, G. *Fedn Proc.* **28**, 1739–1753 (1969).
- Old, L. J. & Boyse, E. A. *A. Rev. Med.* **15**, 167–186 (1964).
- Hardy, W. D. Jr *et al. Science* **166**, 1019–1021 (1969).
- Yoshiki, T., Mellors, R. C. & Hardy, W. D. Jr *Proc. natn. Acad. Sci. U.S.A.* **70**, 1878–1882 (1973).
- Yoshiki, T., Mellors, R. C., Hardy, W. D. Jr & Fleissner, E. *J. exp. Med.* **139**, 925–942 (1974).
- Habel, K. *Fedn Proc.* **22**, 438 (1963).
- Girardi, A. J. *Proc. natn. Acad. Sci. U.S.A.* **54**, 445–451 (1965).
- Kurth, R. & Bauer, H. *Virology* **49**, 145–159 (1972).
- Gelderblom, H., Bauer, H. & Graf, T. *Virology* **47**, 416–425 (1972).
- Hardy, W. D. Jr, Old, L. J., Hess, P. W., Essex, M. & Cotter, S. *Nature* **244**, 266–269 (1973).
- Essex, M., Klein, G., Snyder, S. P. & Harrold, J. B. *Nature* **233**, 195–196 (1971).
- Essex, M. in *Adv. Cancer Res.* **21** (ed Klein, G. & Weinhouse, S.) 175–248 (Academic, New York, 1975).
- Essex, M., Sliski, A., Cotter, S. M., Jakowski, R. M. & Hardy, W. D. Jr *Science* **190**, 790–792 (1975).
- Hardy, W. D. Jr *et al. Cancer Res.* **36**, 582–588 (1976).
- Essex, M., Klein, G., Snyder, S. P. & Harrold, J. B. *Int. J. Cancer* **8**, 384–390 (1971).
- Hardy, W. D. Jr, Hirshaut, Y. & Hess, P. in *Unifying Concepts of Leukaemia* (eds Dutcher, R. M. & Chieco-Bianchi, L.) 778–799 (Karger, Basel, 1973).
- Taylor, D., Hokama, Y. & Perri, S. F. *J. Immun.* **115**, 862–865 (1976).
- Essex, M., Cotter, S. M., Stephenson, J. R., Aaronson, S. A. & Hardy, W. D. Jr in *Origins of Human Cancer* (ed Hiatt, H. H., Watson, J. D. & Winston, J. A.) (Cold Spring Harbor Laboratories, New York, in the press).
- Stephenson, J. R., Essex, M., Hino, S., Hardy, W. D. Jr & Aaronson, S. A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1219–1223 (1977).
- Sliski, A. H., Essex, M., Meyer, C. & Todaro, G. *Science* **196**, 1336–1339 (1977).

## Role of sendai virus fusion-glycoprotein in target cell susceptibility to cytotoxic T cells

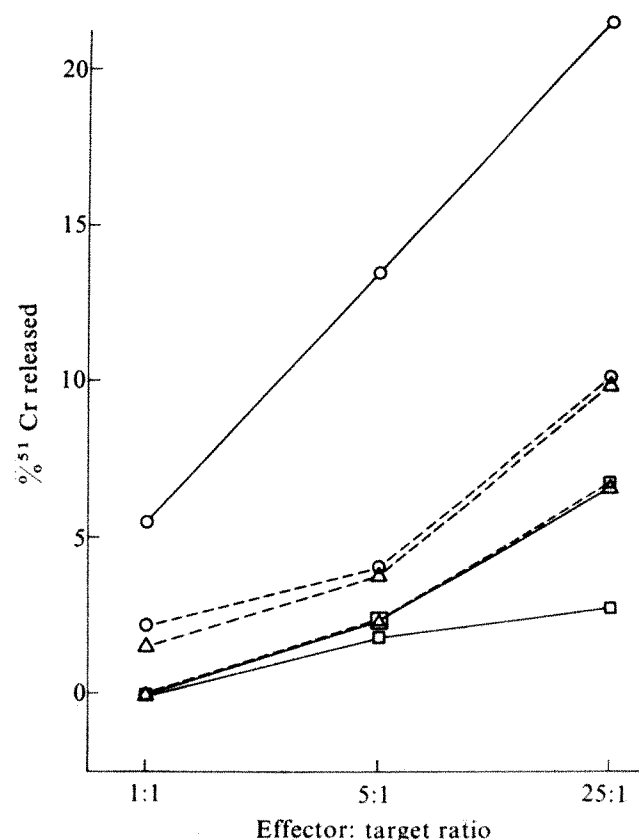
In mice, cytotoxic T lymphocytes (CTLs) are generated *in vivo* or *in vitro* against virally-infected syngeneic cells<sup>1–4</sup>. The specificity of killing requires that the target cells possess both viral gene product(s) and H-2K and/or D gene products which are identical to those on the effector cells<sup>5</sup>. Target cells coated with non-infectious (ultraviolet-inactivated) Sendai virus are also specifically lysed by syngeneic virus-specific CTLs<sup>6</sup>. We show here that it is primarily the fusion glycoprotein of the Sendai virus envelope which is essential for the formation of the target antigen on cells coated with ultraviolet-inactivated Sendai virus.

The Nagoya strain of Sendai virus (from Dr H. Tozawa, Kitasato University, Japan), grown in 10-d-old embryonated chicken eggs, was purified and inactivated by exposure to ultra-

violet light. Ultraviolet-treated Sendai virus (SVuv) shows no infectivity ( $<5$  PFU per ml, no plaques for a 0.2 ml undiluted sample) in primary rhesus monkey kidney cells and also no detectable synthesis of viral proteins in coated P815 (H-2<sup>d</sup>) mouse mastocytoma cells (from Dr B. Smith, Institute for Cancer Research, Philadelphia) at 24 h after coating with virus as measured by direct FITC-antibody (Microbiological Associates), staining (data not shown). For the selective digestion of the fusion glycoprotein of the Sendai viral envelope, SVuv was treated with trypsin  $20 \mu\text{g ml}^{-1}$  according to the method of Shimizu *et al.*<sup>7</sup>.

Table 1 shows that trypsin treatment of SVuv resulted in an 88% decrease in cell-fusion activity and an approximately 95% decrease in haemolytic activity. Thus, trypsin inactivates the Sendai virus fusion and haemolytic activities, both of which reside on the viral fusion glycoprotein (referred to as the F glycoprotein)<sup>8</sup>. On the other hand, trypsin did not affect the viral haemagglutinin (HA) and neuraminidase (NA) activities which are both known to reside on the only other viral glycoprotein (referred to as the HANA glycoprotein)<sup>8</sup> (Table 1). Results similar to these have also been observed by Shimizu *et al.*<sup>9</sup>. Thus, the two ultraviolet-treated Sendai virus preparations used for coating target cells differed in that one possessed the functional activities of the F glycoprotein (referred to as SVf<sup>+</sup>) and the other lacked the functional activities of the F glycoprotein following trypsin digestion (referred to as SVf<sup>-</sup>).

Sendai virus-specific cytotoxic effector cells were prepared using a modification of the method of Schrader *et al.*<sup>6</sup>. DBA/2J (H-2<sup>d</sup>) mouse spleen cells, which had been primed *in vivo* with SVuv, were re-stimulated *in vitro* with  $\gamma$ -irradiated syngeneic spleen cells coated with SVuv. As a control, normal (unprimed) DBA/2J spleen cells were co-cultured with  $\gamma$ -irradiated normal C3H/HeJ (H-2<sup>k</sup>) mouse spleen cells. Only SVf<sup>+</sup> coated syngeneic P815 (H-2<sup>d</sup>) cells were killed significantly by Sendai virus-specific sensitised DBA spleen cells. SVf<sup>-</sup>-coated and mock-infected P815 targets were not killed significantly by the effector cells (Fig. 1). Effector cells stimulated *in vitro* with normal allogeneic (C3H/HeJ) spleen cells did not significantly lyse the P815 target cells (Fig. 1). Therefore, the virus-specific lympholysis was not due to nonspecific killing caused by effector cells binding to target cells by Sendai virus bridges. After the *in vitro* generation, treatment of effector cells with anti-Thy-1.2 and complement showed that SVuv-specific killing was due to T cells (data not shown). These experiments were



**Fig. 1** Susceptibilities of P815 target cells coated with SVf<sup>+</sup> or SVf<sup>-</sup> to Sendai virus-specific CTLs. DBA/2J mice were primed *in vivo* with a single injection of 100–200 haemagglutinating units (HAU) of SVuv per mouse 3 weeks before collection of spleen cells.  $5 \times 10^7$  spleen cells were cultured with  $3 \times 10^7$  unprimed DBA/2J spleen cells pretreated with  $\text{NH}_4\text{Cl}$  to remove red blood cells, coated with SVuv at a concentration of 100–200 HAU per  $1 \times 10^7$  cells (as described below), and  $\gamma$ -irradiated at 2,000 R. Using the procedure described above, unprimed DBA spleen cells were cultured with  $5 \times 10^7$  normal C3H/HeJ spleen cells. The culture medium consisted of EHAA<sup>11</sup> supplemented with  $5 \times 10^{-5}$  M 2-mercaptoethanol and 5% heat-inactivated foetal calf serum. Effector cells were collected 6 d later from cultures containing the virus-coated syngeneic stimulating cells (—) and the allogeneic stimulating cells (---) by using Ficoll-Isopaque fractionation to remove red cells and dead cells<sup>12</sup>. Target cells were prepared as follows: <sup>51</sup>Cr-labelled P815 cells were treated at 4 °C for 1 h with SVf<sup>+</sup> in PBS (○), SVf<sup>-</sup> in PBS (△), or PBS (□) at 200 HAU per  $1 \times 10^7$  cells. The cells were then washed twice with PBS. Cytotoxicity assays were performed in round bottom-wells of a microplate (Linbro) with  $1 \times 10^4$  target cells and a given number of effector cells per well and were allowed to react for 6 h. The data are plotted as % <sup>51</sup>Cr-release for triplicate wells. The indicated s.d. was always less than 5%. The spontaneous release of all targets was 23–26%. Percentage cytotoxicity was calculated as follows:  

$$\frac{\text{c.p.m. experimental release} - \text{c.p.m. spontaneous release} \times 100}{\text{c.p.m. maximum release} - \text{c.p.m. spontaneous release}}$$

**Table 1** Effects of trypsin treatment on Sendai virus envelope activities

Sendai virus	HAU per ml	NAU per ml	% Cell fusion	% Haemolysis
Trypsin treated	1,900	58	12	4.8
Untreated	1,900	56	100	100

Sendai virus grown in embryonated chicken eggs was purified by discontinuous sucrose gradient (20% and 50% (w/w)) centrifugation and resuspended in phosphate-buffered saline (PBS) (pH 7.2). Aliquots of 2 ml of purified virus were irradiated in the ultraviolet at  $800 \mu\text{W cm}^{-2}$  for 30 min with continuous rocking in a plastic dish (Falcon 3002). The ultraviolet-inactivated virus was treated with trypsin (DCC treated bovine pancreatic trypsin, Sigma) at a final concentration of  $20 \mu\text{g ml}^{-1}$  at 36 °C for 30 min. Trypsin was removed by centrifugation at  $55,000g$  for 30 min at 4 °C and the trypsin-treated (SVf<sup>-</sup>) and untreated virus (SVf<sup>+</sup>) were each resuspended in PBS. Haemagglutination (HA), neuraminidase (NA) and haemolytic assays were carried out as described previously<sup>10</sup>. The cell fusion activities of the virus preparations were expressed as the percentage decrease in the total number of cells in the virus-infected cultures (compared to mock-infected control cultures) due to the cell-to-cell fusions. For cell fusion measurements, replicate cultures of HeLa cells (seeded 1 day previously at  $2 \times 10^6$  cells per 60 mm plastic Petri dish) were inoculated with 0.5 ml of SVf<sup>+</sup>, SVf<sup>-</sup>, or mock-infected. Following a 1 h virus adsorption, the cultures were overlaid with 5.0 ml of media containing 10% foetal calf serum. Total cell numbers were measured 2 h later.

repeated three times with identical results to those shown in Fig. 1. Thus, the Sendai virus F glycoprotein is the primary virus component necessary for the lysis of ultraviolet-inactivated virus-coated target cells by syngeneic CTLs.

Treatment of the Sendai virion with trypsin causes selective proteolytic cleavage(s) of the F glycoprotein<sup>7</sup> and inactivates its biological activities of fusion and haemolysis (Table 1 and ref. 9) with no detectable effect on the amount of viral HANA glycoprotein on the virus-coated cells. Table 2 shows that there is no significant difference in the cell-associated NA activities between SVf<sup>+</sup>- and SVf<sup>-</sup>-coated cells for at least 6 h after virus coating, which corresponds exactly to the cytotoxicity reaction time (Fig. 1). Similar results to those in Table 2 were obtained in a repeat experiment. These experiments rule out the possi-

**Table 2** Neuraminidase activities of Sendai virus-coated P815 target cells

Time after coating cells (h)	Neuraminidase activity ( $A_{540}$ ) from cells coated with		
	SVf <sup>+</sup>	SVf <sup>-</sup>	No virus
0	0.462	0.491	0.112
3	0.229	0.326	0.112
6	0.223	0.275	0.111

P815 cells were treated with SVf<sup>+</sup> in PBS, SVf<sup>-</sup> in PBS, or PBS in the conditions described for the labelled target cell preparations (see Fig. 1 legend). After washing, the cells were incubated at 37 °C in the culture medium for the indicated times. Following incubation,  $2.4 \times 10^6$  cells were pelleted and assayed for cell-associated viral neuraminidase activity using the assay method used for Sendai virions<sup>10</sup>.

bility that there is a difference in the amount of adsorbed viral HANA glycoprotein on the target cells coated with either SVf<sup>+</sup> or SVf<sup>-</sup>, that is, that fusion activity determines the amount of virus adsorbed to the cell surface in these conditions.

During the course of these studies, Koszinowski *et al.*<sup>13</sup> reported that target cells coated with a partially purified envelope fraction (containing both HANA and F glycoproteins from disrupted Sendai virions) were susceptible to syngeneic virus-specific CTLs. Thus, our studies confirm and extend these results by showing that it is the F glycoprotein of the Sendai virus envelope that has an essential role in the formation of the target antigen on the inactivated virus-coated target cells recognised by the syngeneic (ultraviolet-inactivated Sendai virus-specific) CTLs.

Two general hypotheses have been proposed to explain H-2 restriction in virus-specific lymphocyte cytotoxicity<sup>14</sup>. The CTLs could recognise the H-2K and/or D gene products which are modified by the virus (altered-self hypothesis) or the CTLs could recognise both H-2 and viral gene products (dual recognition hypothesis). Recently, Ennis *et al.*<sup>15</sup> reported experiments which support the dual recognition of both H-2 and viral haemagglutinin antigens for influenza virus-specific CTLs. It is not known whether the H-2 and viral antigens were recognised by two separate receptors, one for the H-2 antigen and the other for the influenza virus haemagglutinin antigen, or whether there could be one receptor specific for some structural association between these two antigens<sup>14</sup>. A possible structural association of H-2 and murine leukaemia virus envelope glycoprotein on the surface of mouse tumour cells producing murine leukaemia virus has been suggested from co-capping and co-patching studies<sup>16</sup>.

There are several possible ways of explaining the role of the F glycoprotein in the Sendai virus-specific cytotoxic reaction. The fusion activity of the ultraviolet-inactivated Sendai virus may be required for an association of H-2 gene products and virion component(s). It is believed that Sendai virion components can disperse quickly throughout the cell membrane after fusion of the viral envelopes<sup>17</sup>. Following the dispersion of Sendai virion components, the F glycoprotein itself and/or other viral gene products could interact with H-2 gene products in the cell membrane. Alternatively, the fusion glycoprotein itself could directly become a part of the target antigen without the expression of its fusion activity.

Experiments are in progress to discriminate between these various possibilities by using purified subcomponents isolated from Sendai virions. We are also investigating the possibility that in conditions where cells are infected with live (not ultraviolet-inactivated) viruses other virion or viral-induced component(s) could also contribute to the formation of the target antigens recognised by syngeneic virus-immune CTLs.

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1. Zinkernagel, R. M. & Doherty, P. C. *Nature* **248**, 701-702 (1974).
2. Koszinowski, U. & Thomssen, R. *Eur. J. Immun.* **5**, 245-251 (1975).
3. Doherty, P. C. & Zinkernagel, R. M. *Immunology* **31**, 27-32 (1976).
4. Dunlop, M. B. C. & Blanden, R. V. *Cell. Immun.* **28**, 190-197 (1977).
5. Doherty, P. C., Blanden, R. V. & Zinkernagel, R. M. *Transplant. Rev.* **29**, 89-124 (1976).
6. Schrader, J. W. & Edelman, G. M. *J. exp. Med.* **145**, 523-539 (1977).
7. Shimizu, K. & Ishida, N. *Virology* **67**, 427-437 (1975).
8. Hosaka, Y. & Shimizu, K. in *Virus Infection and the Cell Surface* 129-155 (eds Poste, G. & Nicolson, G. E.) (North-Holland, Amsterdam, 1977).
9. Shimizu, K., Shimizu, Y. K., Yasuda, H. & Ishida, N. *Biophysics (Japan)* **17**, 20-28 (1977).
10. Shimizu, K., Hosaka, Y. & Shimizu, Y. K. *J. Virol.* **9**, 842-850 (1972).
11. Peck, A. B. & Bach, F. H. *J. Immun. Meth.* **3**, 147-164 (1973).
12. Goldmann, S. F. & Flad, H. D. *Tissue Antigens* **5**, 145-154 (1975).
13. Koszinowski, U., Gething, M. J. & Waterfield, M. *Nature* **267**, 160-163 (1977).
14. Doherty, P. C., Götze, D., Trinchieri, G. & Zinkernagel, R. M. *Immunogenetics* **3**, 517-524 (1976).
15. Ennis, F. A., Martin, W. J., Verbonitz, M. W. & Butchko, G. M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3006-3010 (1977).
16. Schrader, J. W., Cunningham, B. A. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5066-5070 (1975).
17. Howe, C. & Morgan, C. J. *J. Virol.* **3**, 70-81 (1969).

## Isolation of separate Fc receptors for IgG complexed to antigen and native IgG from a murine leukaemia

RECENT studies have suggested that membrane receptors for the Fc region of IgG (Fc receptors) may be heterogeneous structures with distinctive binding properties<sup>1-5</sup>. Walker has reported that a single cell line may possess separate Fc receptors for IgG molecules of different subclass<sup>1</sup>, and Heusser *et al.* have demonstrated that macrophage-like cell lines may possess two distinct receptors: one which binds monomeric IgG2a, and another which binds to aggregates of all subclasses<sup>2</sup>. Unkeless recently reported that a variant clone of the P388D1 mouse macrophage line had the same number of binding sites for IgG2a as the parent line, but only 10% of the binding sites for rabbit IgG in the form of soluble antigen-antibody complexes, suggesting the presence of two distinct Fc receptors<sup>3</sup>. The molecular identity of Fc receptors remains uncertain since few structural studies defining these membrane components are currently available<sup>6-8</sup>. In this study we demonstrate that several glycoproteins with Fc binding activity can be isolated from the plasma membranes of L1210 cells, and provide structural evidence that separate receptors may be responsible for binding IgG which has been complexed with antigen, and monomeric or aggregated IgG.

The murine leukaemia L1210 was grown in stationary culture in RPMI 1640 supplemented with 10% foetal calf serum (Flow Laboratories, Rockville, Maryland). Cells ( $2 \times 10^{10}$ ) were swelled in hypotonic buffer and ruptured in a Ten Broeck homogeniser. The plasma membranes were prepared by a partition of the cell lysates in a two-phase system of polyethylene glycol and dextran<sup>9</sup>. The glycoproteins were extracted from the plasma membranes using 0.3 M lithium diiodosalicylate as described by Marchesi and Andrews<sup>10</sup>. In some experiments, the cells were labelled with <sup>125</sup>I using lactoperoxidase-catalysed iodination before lysing, and in other experiments the glycoproteins were labelled with <sup>125</sup>I by the chloramine-T method<sup>11</sup> after the extraction. In the two experiments in which lactoperoxidase-catalysed iodination was used, 39% and 46% of the cell-associated counts were recovered in the plasma membrane interphase, and 43% of the plasma membrane-



associated counts were recovered in the glycoprotein extract. The results obtained using the two labelling techniques were identical.

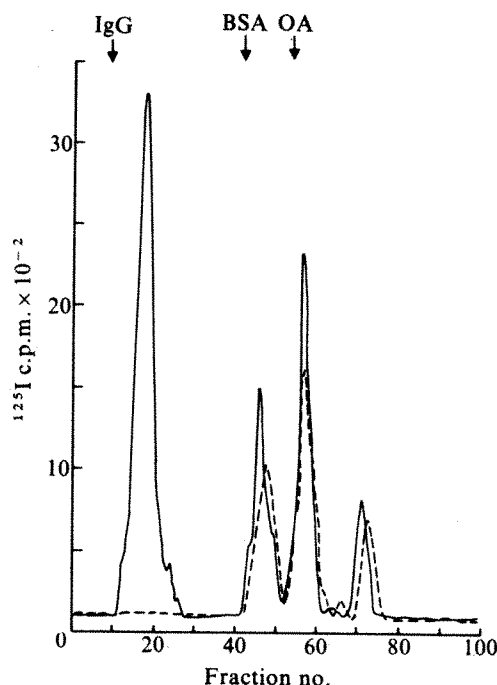
The glycoprotein extract obtained from  $2 \times 10^{10}$  cells contained approximately 2 mg protein and seemed to have Fc receptor activity since it was bound by erythrocytes coated with IgG antibody, but not by erythrocytes alone. The addition of 1  $\mu$ g of the glycoprotein extract to  $10^6$  L1210 cells and  $10^8$  IgG-sensitised erythrocytes resulted in 73% inhibition of EA rosette formation.

Purified human IgG (Miles Laboratories, Elkhart, Indiana) was extensively absorbed with viable L1210 cells and an F(ab')<sub>2</sub> fraction prepared by pepsin digestion and Sephadex G-200 fractionation<sup>12</sup>. Purity of the intact and digested IgG was determined using SDS-polyacrylamide gel electrophoresis<sup>13</sup>. Cyanogen bromide-activated Sepharose 4B was coupled to either heat-aggregated human IgG, heat-aggregated F(ab')<sub>2</sub> human IgG, or keyhole limpet haemocyanin (KLH) as described previously<sup>6</sup>, and five columns containing 2 mg of coupled protein were prepared. Rabbit anti-human IgG (Cappel Laboratories, Downingtown, Pennsylvania) was added to one of the columns containing coupled aggregated human IgG, and rabbit anti-KLH was added to one of the KLH-coupled columns, so that two columns contained antibody complexed to antigen. The other columns contained only coupled KLH, aggregated human IgG or aggregated F(ab')<sub>2</sub> IgG. All columns were washed with phosphate-buffered saline (PBS) until the  $A_{280}$  in the eluate was equal to a blank control.

Equal amounts of radioactive glycoprotein extract in PBS were applied to each column and after a 60-min incubation at 23 °C the columns were washed with PBS until the c.p.m. in the eluates were equal to background. The columns and washes were counted and the percentage c.p.m. bound was calculated (Table 1). Those columns containing Sepharose 4B coupled to IgG with an intact Fc region bound between 35–44% of the labelled preparation, whereas the F(ab')<sub>2</sub> IgG and KLH columns bound only 2.8% and 4.4%, respectively. Elution of the columns with 0.1 M citrate buffer (pH 3.2) containing 0.5 M NaCl removed most of the radioactive material which was bound to the columns that contained an intact Fc region (Table 1).

The citrate eluates were analysed using SDS-polyacrylamide gel electrophoresis (Fig. 1). No distinct components were detected in the citrate eluates from the F(ab')<sub>2</sub> IgG-coupled or the KLH-coupled columns. Three distinct peaks with apparent molecular weights of 65,000, 45,000 and 28,000 (designated FI, FII and FIII) were found in the eluate from the aggregated IgG-coupled column (Fig. 1). An identical pattern was observed when non-aggregated IgG was coupled to Sepharose 4B. The citrate eluates from the columns containing immune complexes (IgG-anti-IgG or KLH-anti-KLH) revealed the same three peaks and an additional peak (designated peak A) with an apparent molecular weight of 125,000 (Fig. 1). The possibility that peak A represents a gel artefact due to overloading with IgG eluted from the complex columns was discounted by the fact that only a faint Coomassie blue-positive IgG band was present on the gels. Furthermore, gels loaded with one tenth the radioactive material revealed no IgG staining band, but the continued presence of peak A.

Peak A, FI, FII and FIII were purified by electroelution from polyacrylamide gels<sup>14</sup> and each fraction contained less than 8%



**Fig. 1** Analyses of the SDS-polyacrylamide gel electrophoreses of the citrate eluates derived from Sepharose 4B columns coupled to aggregated human IgG to which anti-human IgG had been added (—), or to aggregated human IgG alone (---). The radioactive samples, in non-reducing conditions, were applied to parallel 7.5% acrylamide gels. Electrophoreses were carried out in non-reducing conditions to maintain the Fc binding properties of the proteins which were subsequently purified from the gels by electroelution. The citrate eluate from the KLH-coupled column, to which anti-KLH had been added, revealed an identical profile to the aggregated IgG-anti-IgG column. The citrate eluate from a non-aggregated IgG-coupled column had the same pattern as the aggregated IgG-coupled column. Peak A, which was demonstrated only in the citrate eluate of columns containing immune complexes, migrates in front of IgG and has an apparent molecular weight of 125,000. FI, FII, and FIII, with apparent molecular weights of 65,000, 45,000 and 28,000, were found in the citrate eluates from all columns that contained an intact Fc region. The citrate eluates from the F(ab')<sub>2</sub> IgG-coupled and KLH-coupled columns revealed no distinct peaks.

cross contamination from the other fractions. FI, FII and FIII revealed no change in molecular weight after reduction and alkylation. Reduction and alkylation of peak A resulted in the appearance of two radioactive peaks with apparent molecular weights of 74,000 and 60,000.

The ability of non-aggregated mouse myeloma proteins of different IgG subclasses to bind to intact L1210 cells was determined by indirect immunofluorescence. As judged by the intensity of the fluorescence, Table 2 shows that L1210 binds IgG preparations in the following order: human IgG = mouse IgG2a > mouse IgG1 > mouse IgG2b. Affinity columns of Sepharose 4B coupled to these same proteins were prepared and the percentage labelled peak A, FI, FII, and FIII bound by these columns determined (Table 2). All isolated glycoproteins bound better to human IgG or mouse IgG2a than to mouse IgG1 or IgG2b.

**Table 1** Binding of <sup>125</sup>I-labelled glycoprotein extract to Sepharose 4B columns coupled to different IgG preparations

Buffer	Aggregated F(ab') <sub>2</sub> IgG	Aggregated IgG	% c.p.m. bound Aggregated IgG + anti-IgG	KLH	KLH + anti-KLH
PBS, 0.01 M (pH 7.2)	2.8	35.7	44.1	4.4	42.7
Citrate, 0.1 M NaCl, 0.5 M (pH 3.2)	1.6	8.4	6.7	4.2	10.1

Equal amounts of radioactive glycoprotein extract in PBS were applied to the columns. After extensive washing, so that the counts in the eluate equalled background, the columns and washes were counted and the percentage of the applied c.p.m. bound was calculated. The columns were then eluted with citrate buffer and the percentage of applied c.p.m. remaining on the columns was calculated.

**Table 2** Correlation of IgG subclass binding of intact L1210 cells and isolated Fc binding proteins

Sample	Human IgG	Mouse myeloma proteins		
		IgG1	IgG2a	IgG2b
L1210 Cells	++++	++	++++	+
Peak A	74.8	32.8	61.2	26.2
FI	59.6	40.8	63.8	32.6
FII	66.6	26.9	64.9	35.6
FIII	45.5	30.4	46.6	30.0

L1210 cells were incubated with either human IgG or one of the mouse myeloma proteins, washed, and then stained with the appropriate fluorescein conjugate (anti-human IgG or anti-mouse Ig). The intensity of the fluorescence was graded from + (barely visible) to ++++ (most intense). Peak A, FI, FII and FIII were passed over Sepharose 4B columns to which equal amounts of human IgG or one of the myeloma proteins had been coupled. The columns were washed with PBS until the c.p.m. in the eluates were equal to background. The numbers refer to the percentage of counts bound by each column.

These experiments indicate that four proteins which bind to the Fc region of IgG can be isolated from the glycoprotein extract of the plasma membranes of L1210. These proteins exhibit the same IgG subclass affinity as the intact cell, indicating their relationship to the cell's Fc receptors; however, their relationship to one another has not been determined. FI, FII, and FIII are composed of single polypeptide chains of similar amino acid composition, yet they differ considerably in their apparent molecular weights. Preliminary data suggest that the apparent differences may be due to either differences in the carbohydrate moieties of the molecules, or aggregation which is induced during the isolation procedure on SDS gels<sup>15</sup>. The smaller proteins may also represent degradation products of a larger Fc receptor. We consider this possibility less likely, since the addition of proteolytic inhibitors throughout the isolation procedure does not alter the results, and the isolated molecules still retain their Fc-binding properties.

Frøland *et al.*<sup>4</sup> have presented data which suggest that the cells that form EA rosettes and those that bind aggregated IgG may be separate. Our data may provide a molecular basis for explaining these observations. The Fc binding proteins (FI, FII and FIII) isolated from L1210 bind to monomer or heat-aggregated IgG, whereas peak A seems to have specificity for IgG complexed to antigen. Thus, the ability to form rosettes with IgG-coated erythrocytes could depend on the possession of an Fc receptor which binds IgG antibody complexed to antigen, and this receptor may be distinct from the Fc receptor which binds IgG that is not complexed to antigen. It is also possible that the smaller Fc binding molecules which we have isolated may be related to each other and may be derived from a larger, complex Fc receptor. The different binding specificities exhibited by an individual cell could then be accounted for by the way in which these molecules are arranged or linked to one another on the cell's surface.

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- Walker, W. S. *J. Immun.* **116**, 911-914 (1976).
- Heusser, C. H., Anderson, C. L. & Grey, H. M. *J. exp. Med.* **145**, 1316-1327 (1977).
- Unkeless, J. C. *J. exp. Med.* **145**, 931-945 (1977).
- Frøland, S. S., Natvig, J. B. & Michaelsen, T. E. *Scand. J. Immun.* **3**, 375-380 (1974).
- Lobo, P. I. & Horwitz, D. A. *J. Immun.* **117**, 939-943 (1976).
- Cooper, S. M. & Sambray, Y. *J. Immun.* **117**, 511-517 (1976).
- Rask, L., Klæreskog, L., Ostberg, L. & Peterson, P. A. *Nature* **257**, 231-233 (1975).
- Anderson, C. L. & Grey, H. M. *J. Immun.* **118**, 819-825 (1977).
- Hourani, B. T., Clance, N. H. & Pincus, J. H. *Biochem. biophys. Acta* **328** 520-532 (1973).
- Marchesi, V. T. & Andrews, E. P. *Science* **174**, 1247-1248 (1971).
- Hunter, W. M., Greenwood, R. C. & Glover, J. S. *Biochem. J.* **89**, 114-124 (1963).
- Stanworth, D. R. & Turner, M. W. in *Handbook of Experimental Immunology*, second edition (ed. Weir, D. M.) 10.1-10.97 (Blackwell Scientific, Oxford, 1973).
- Laemmli, U. K. *Nature* **227**, 680-685 (1970).
- Benay, P. D., Padilla, S. R. & Nimni, M. E. *Biochemistry* **16**, 865-872 (1977).
- Cooper, S. M. & Sambray, Y. *J. supramolec. Struct.* (in press).

## Antagonism of antigen-induced contraction of guinea pig and human airways

EXPOSURE of isolated sensitised guinea pig or human airway tissue to specific antigen produces a rapid and prolonged contraction which has been studied as a model of asthmatic bronchospasm. This response is thought to be mediated by endogenous chemicals released from airway mast cells, since antigen treatment of sensitised guinea pig or human pulmonary tissue results in the elaboration of several substances, including histamine and slow reacting substance of anaphylaxis (SRS-A), which can contract airway smooth muscle. The few attempts at investigation of which mediators are responsible for the airways response have led to contradictory conclusions. Schild *et al.*<sup>1</sup> reported that antihistamines significantly (but not completely) antagonised antigen-induced contraction of bronchi from an asthmatic patient. Sheard and Blair<sup>2</sup>, however, demonstrated that antihistamines had no effect on the contraction of human bronchial strips studied in the presence of antigen-challenged, passively-sensitised human lung. They suggested, without direct evidence, that the contractile response of the bronchial strip was the result of SRS-A release. At present, therefore, the roles of histamine and SRS-A in the airway response to antigen have not been defined. We have investigated the effects of diphenhydramine, a histamine H<sub>1</sub> antagonist, and FPL 55712 (ref. 3), an SRS-A antagonist, on the response of sensitised guinea pig and human airways to antigen and report that pretreatment with either alone modifies the airway response in a characteristic fashion, and that together, they effectively inhibit antigen-induced airway constriction.

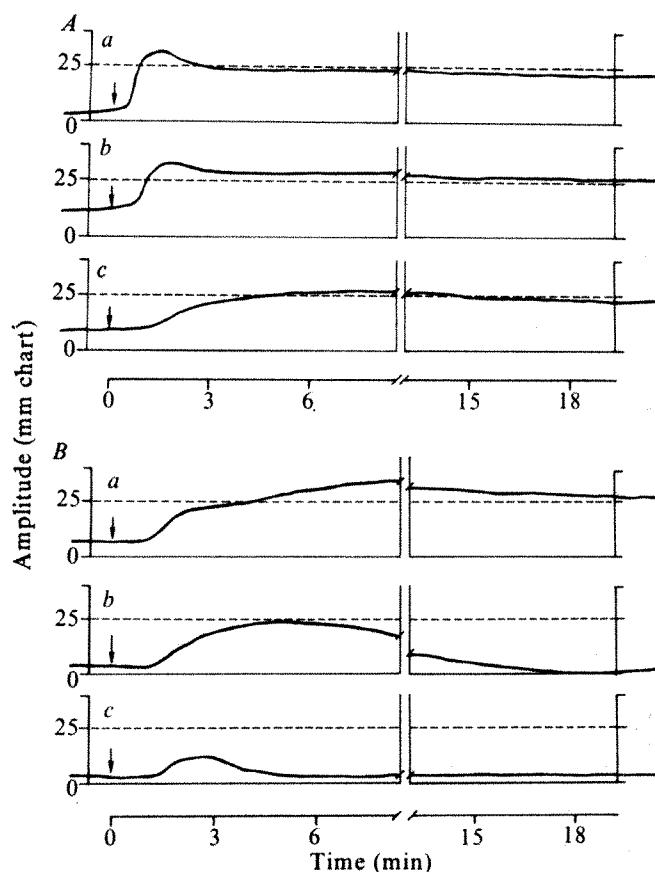
Tracheas of male Hartley guinea pigs passively sensitised with rabbit anti-dog albumin were cut into rings which were tied together to form a chain and placed in an organ bath containing Tyrode's solution. Three chains of three rings each were constructed from each trachea and were studied simultaneously. Human bronchial tissue obtained from patients undergoing surgical procedures was sensitised by incubating with serum from a ragweed allergic subject. Circumferential bronchial strips, studied in duplicate or triplicate, were suspended in an organ bath containing Krebs-Henseleit solution. Isometric airway tissue contractions were recorded.

Exposure of either tissue to specific antigen resulted in a rapid contraction equivalent in amplitude to approximately 40-100% of maximum contraction. Unsensitised tissue did not respond. Although the amplitude of the antigen-induced contraction varied considerably from day to day, within each experiment the responses of the chains or strips from one smooth muscle preparation were approximately equal. The duration of the response, ranging from 30 to 150 min, was proportional to the amplitude and was essentially unaffected by repeated washings.

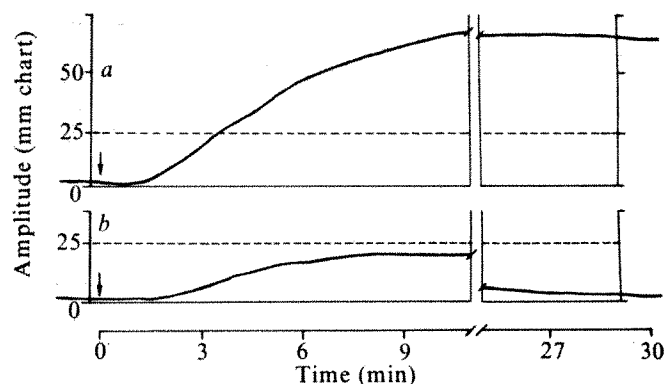
Pretreatment of guinea pig or human airway tissue with diphenhydramine delayed the onset of the response to antigen and decreased the rate of development of the contraction (Fig. 1A). Within each experiment, the effects of diphenhydramine were dose dependent, with effects noted at concentrations as low as 10<sup>-7</sup> M. Pretreatment with doses of diphenhydramine as high as 10<sup>-4</sup> M, however, did not significantly decrease the amplitude or duration of the antigen-induced contraction. Higher concentrations caused no specific effects, but even 10<sup>-3</sup> M diphenhydramine had no effect when added after the airway response to antigen had developed.

Pretreatment with FPL 55712 in doses up to 5 × 10<sup>6</sup> g ml<sup>-1</sup> did not significantly alter the initial phase of the airways

response to antigen but produced a dose-related decrease in amplitude and duration of the prolonged phase of the response (Fig. 1*B,b*). Higher concentrations of FPL 55712, in addition to further depressing the amplitude and duration of the prolonged phase, inhibited to some extent the initial phase of the antigen-induced contraction



**Fig. 1** Effect of, *A*, diphenhydramine, and *B*, FPL 55712, on the response of sensitized guinea pig trachea to antigen. *A*, Male Hartley guinea pigs (300–350 g) were passively sensitized by injecting 0.2 ml rabbit anti-dog albumin (Cappel Laboratories, Cochranville, Pennsylvania, 2 mg antibody protein ml<sup>-1</sup>) intraperitoneally (i.p.) 12–16 h before use. After the animals were killed (pentobarbital, 90 mg kg<sup>-1</sup> i.p.), the tracheas were removed and cut into rings 2 mm wide. These were tied together to form a chain and placed in a 37 °C organ bath containing Tyrode's solution bubbled with 95% O<sub>2</sub>–5% CO<sub>2</sub>. Three chains of three rings each were constructed from each trachea and were studied simultaneously. Airway tissue contractions were measured with Grass FT03C force-displacement transducers and recorded on a Grass Model 7 polygraph. After the initial tension was adjusted (1 g), the tissue was washed and treated with several doses of methacholine (2.5 × 10<sup>-6</sup> M), until a consistent response was established. This dose of methacholine produced a contraction of the guinea pig airway tissue approximately equivalent to 75% of maximum, defined in our experiments as the response to 10<sup>-3</sup> M methacholine. Amplifier gain was adjusted to give equivalent recorded contraction amplitude for each chain to the test dose of methacholine. The tissue was then washed and incubated for 10 min with diphenhydramine (*b*, 10<sup>-3</sup> M; *c*, 10<sup>-5</sup> M). *a*, Untreated control tissue. Dog albumin (10<sup>-6</sup> g ml<sup>-1</sup>) was added to each bath at the arrow. Control response to antigen was equivalent to 56% maximum contraction. Diphenhydramine produced a delay in onset of response to antigen (100% greater than control for 10<sup>-7</sup> M, 200% for 10<sup>-5</sup> M) and a decrease in initial rate of contraction. Duration of response (120 min) was unaffected. *B*, Tissue preparation as *A*, except that tracheal rings were incubated with FPL 55712 for 10 min (*b*, 2 × 10<sup>-6</sup> g ml<sup>-1</sup>; *c*, 2 × 10<sup>-5</sup> g ml<sup>-1</sup>). *a*, Control response of untreated tissue. Dog albumin (10<sup>-6</sup> g ml<sup>-1</sup>) was added to each of the baths at the arrow. Control response to antigen was equivalent to 60% maximum contraction. In contrast to antihistamine (*A*), FPL 55712 decreased duration of response to antigen by 85% (2 × 10<sup>-6</sup> g ml<sup>-1</sup>) and 95% (2 × 10<sup>-5</sup> g ml<sup>-1</sup>). Control duration was 110 min. Decrease in amplitude of early phase of response (*c*) was a result of antihistamine-like properties of FPL 55712.



**Fig. 2** Effect of diphenhydramine in combination with FPL 55712 on sensitized human airways response to antigen. Human bronchial tissue, obtained from a patient undergoing surgery, was passively sensitized by incubating overnight at 25 °C with serum from a ragweed allergic subject (1:40 dilution in a balanced salt solution). Serial circumferential subsegmental bronchial strips were suspended in organ baths containing Krebs–Henseleit solution, bubbled with 95% O<sub>2</sub>–5% CO<sub>2</sub> and maintained at 37 °C. Tissue contractions were measured as described in Fig. 1. After a consistent response to methacholine (2.5 × 10<sup>-6</sup> M) was established and amplifier gain was adjusted to give equivalent recorded contractions to the test dose of methacholine, the tissue was washed and the bronchial strip depicted in *b* was incubated with diphenhydramine (10<sup>-3</sup> M) combined with FPL 55712 (10<sup>-5</sup> g ml<sup>-1</sup>) for 10 min. *a*, Control response of untreated tissue. Antigen E (5 × 10<sup>-7</sup> g ml<sup>-1</sup>) was added to each of the baths at the arrow. Control response to antigen was equivalent to 84% maximum contraction. Combination of antagonists produced delay in onset of response, decrease in amplitude of early and prolonged phases of contraction and a decrease in duration. Control response duration was 150 min.

(Fig. 1*B,c*). This effect is attributable to the reported<sup>3,4</sup> antihistamine activity of FPL 55712. FPL 55712 added during the prolonged phase of antigen-induced contraction of guinea pig or human airway tissue reversed the response, the dose required being somewhat higher than that effective in pretreatment.

Diphenhydramine (10<sup>-3</sup> M) in combination with FPL 55712 (10<sup>-5</sup> g ml<sup>-1</sup>) administered before the antigen regularly reduced both the amplitude and duration of the antigen-induced contraction to less than 30% of control, with complete blockade frequently being observed. Figure 2 shows the effect of pretreatment with both diphenhydramine and FPL 55712 on the response of human bronchial tissue to antigen. The extent of inhibition by this combination of antagonists depended on the relative amplitude of the contraction.

These data represent the first clear evidence that both histamine and SRS-A contribute to antigen-induced contraction of sensitized airway tissue and have important implications for the pharmacological control of human asthma. Histamine is primarily involved in the early phase of the response, commencing 1–2 min following the administration of antigen. This is the period most frequently examined in screening drugs by antigen broncho-provocation in man. The effect of histamine was particularly obvious in the pattern of contraction of guinea pig trachea, where the response frequently showed two amplitude maxima, one occurring within 3 min of antigen administration and the other occurring after about 10 min. Pretreatment with diphenhydramine selectively abolished the early contraction maximum without altering the amplitude or duration of the later phase of the response. Maximal inhibition of the early phase of the response required about 10<sup>-3</sup> M diphenhydramine. From data on the antagonism of exogenous histamine it seems that the mean effective tissue concentration of histamine in the region of the smooth muscle cells must be quite high, probably approaching 10<sup>-3</sup> M.

SRS-A seems to contribute to the later, prolonged phase of the airway response to the antigen, since this phase

is effectively antagonised by FPL 55712 but not by diphenhydramine. The dosages of FPL 55712 required to produce a maximal effect were considerably higher than those reported by Augstein<sup>3</sup> or Orange<sup>4</sup> to inhibit SRS-A-induced contraction of guinea pig ileum. This is not surprising since, like that of histamine, the tissue concentration of SRS-A following antigen treatment must be many orders of magnitude higher than that achieved by the addition of exogenous SRS-A to an organ bath. Whether other mediators contribute to the response or modulate the airways reactions to histamine or SRS-A is not yet clear, but it seems evident that pharmacological control of human airways reactions *in vivo* will require either a drug which inhibits the release of multiple mediators or a combination of specific antagonists.

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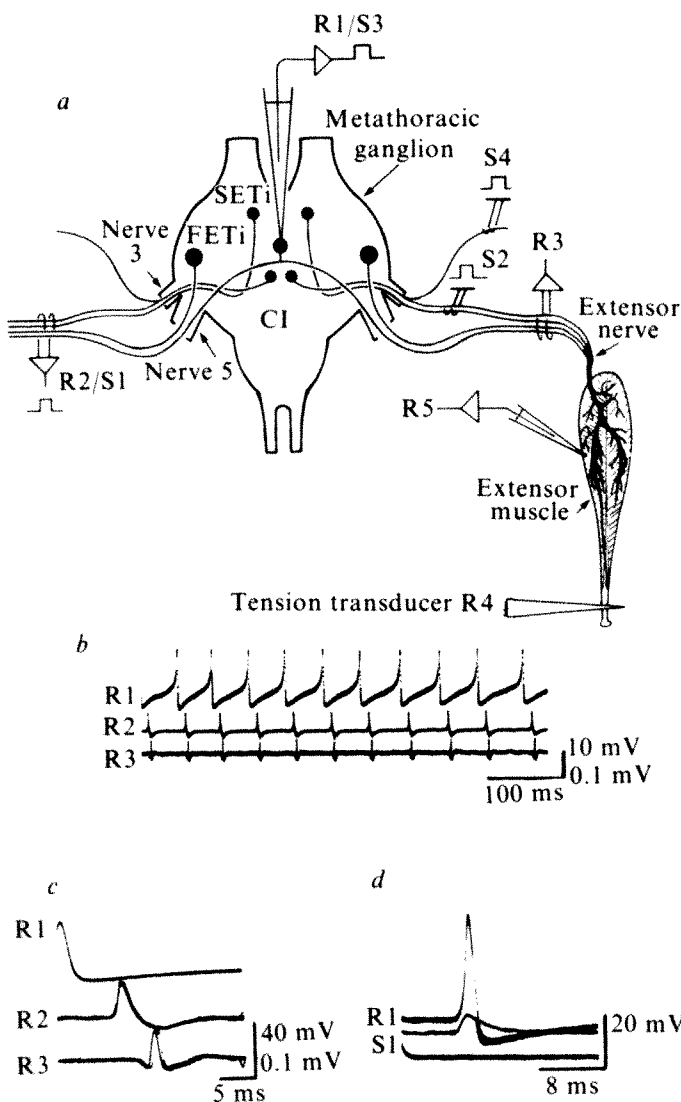
1. Schild, H. O., Hawkins, D. F., Mongar, J. L. & Herxheimer, H. *Lancet* ii, 376-382 (1951).
2. Sheard, P. & Blair, A. M. J. N. *Int. Arch. Allerg. appl. Immunol.* 38, 217-224 (1970).
3. Augstein, J., Farmer, J. B., Lee, T. B., Sheard, P. & Tattersall, M. L. *Nature* 245, 215-217 (1973).
4. Orange, R. P. in *The Role of Immunological Factors in Infectious, Allergic and Autoimmune Processes* (eds Beers, R. F. jun. & Bassett, E. G.) 223-235 (Raven, New York, 1976).

## An octopaminergic neurone modulates neuromuscular transmission in the locust

OCTOPAMINE, a biogenic amine, occurs widely in the nervous systems of vertebrates and invertebrates<sup>1-3</sup>. It has been found in single neurones in the central nervous system of the marine mollusc, *Aplysia*<sup>4</sup> and also in peripheral neurosecretory neurones in the lobster<sup>5,6</sup> from which a calcium-dependent release of octopamine has been demonstrated<sup>7,8</sup>. Specific octopamine receptors<sup>9</sup> and octopamine activated adenylate cyclases<sup>10</sup> have also been found in invertebrate nervous tissue. This has led to the suggestion of a neurotransmitter role for octopamine in invertebrates<sup>1,3</sup>. In the absence, however, of an example in which the effect of stimulating an identified octopamine containing neurone on its identified target or end organ is known, the true function of octopamine remains obscure. This paper aims to demonstrate that octopamine is the neurotransmitter of a single identified neurone in the locust *Schistocerca americana gregaria*. This neurone, which is shown to contain octopamine, innervates a muscle where it functions as a neuromodulator by potentiating the synaptic potential and tension produced by an identified motoneurone. That this function is mediated by the release of octopamine on stimulation is supported by the action of exogenously applied octopamine.

The extensor-tibiae muscle of the locust hind leg is a large and powerful muscle used in walking, kicking and jumping. It is innervated by three motoneurones<sup>11-13</sup> whose axons are contained in the extensor-tibiae nerve. They are the fast and the slow extensor motoneurones (FETi and SETi) and an inhibitory motoneurone which innervates many muscles and has, therefore, been called the common

inhibitor (CI). These neurones are paired and so there are six identified motoneurones controlling the paired (left and right) extensor muscles. Their cell bodies lie in known positions in the metathoracic ganglion (Fig. 1a). The muscle also receives a fourth axon<sup>14</sup> belonging to a single unpaired



**Fig. 1** The experimental arrangement and the identification of DUMETi. *a*, Recording (R) and stimulating (S) sites referred to here and in Fig. 3. ●, Positions of the neurone cell bodies in the locust metathoracic ganglion which have axons in the extensor tibiae nerve. Independent stimulation of each axon in the extensor nerve was achieved as follows. The DUM axon was stimulated either by passing current into the soma of DUMETi (S3) or by antidromic stimulation of the left extensor nerve (S1). Selective stimulation of the axon of the slow extensor motoneurone (SETi) in a branch of nerve 3 (S2) which contains both its axon and that of the common inhibitory motoneurone (CI) is achieved by the relatively larger diameter of the former which gives it a lower threshold to extracellular stimulation. The common inhibitory motoneurone was stimulated in a branch of nerve 3 (S4) which contains its axon but lacks other axons which project to the extensor muscle. Muscle tension is monitored almost isometrically with a tension transducer placed on the muscle tendon (R4). *b*, Spikes initiated in the soma of DUMETi (R1/S3) are transmitted 1:1 to both left and right extensor nerves. This distinguishes the DUMETi neurone soma from those of other DUM cells. *c*, The oscilloscope is triggered several times by spikes recorded in the soma of DUMETi, note that spikes on the left (R2) and right (R3) axons superimpose. The difference in absolute latency between spikes recorded left and right is caused by a difference in the distance from the soma of sites R2 and R3. *d*, The soma spike can be initiated antidromically by stimulating either left or right extensor nerves. If the soma is hyperpolarised, the active soma spike fails to initiate in response to antidromic stimulation (R1, lower trace). The smaller positive potential seen in the soma is a recording of the axon spike conducted passively from its site of initiation.

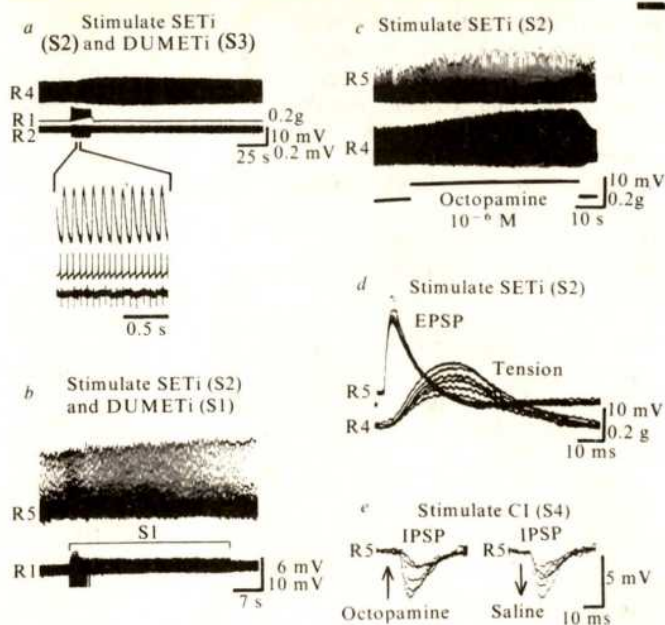
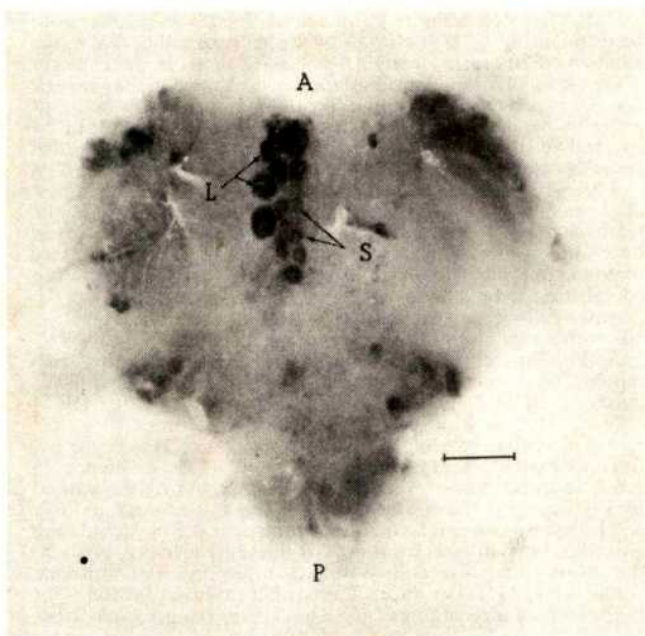


neurone with its cell body among a group of similar unpaired neurones in the dorso-medial region of the metathoracic ganglion<sup>15,16</sup>. They are called Dorsal Unpaired Median or DUM neurones and the one which innervates the extensor-tibiae muscle is known as DUMETi<sup>14</sup>.

The DUM cells stain selectively with the dye neutral red (Fig. 2), a dye known to stain monoamine-containing neurones in the leech<sup>17</sup> and lobster nervous systems<sup>5,6</sup>. This finding, together with the observation that tissue removed from the dorsal part of the locust metathoracic ganglion, possibly containing DUMETi, is capable of octopamine synthesis from tyrosine<sup>18</sup>, directed our attention towards the identification of a biogenic amine, possibly octopamine, as a neurotransmitter for DUMETi. We identified the DUMETi neurone by intracellular recording (Fig. 1 b-e) in 50 animals. The soma was removed, cleaned of adhering pigment cells and other tissue and transferred to a microtube containing 10  $\mu$ l of 0.01M formic acid. Five assays were performed on pools each containing 10 identified DUMETi cell bodies. Octopamine was assayed by a modification (P.D.E. in preparation) of the standard radio-enzymatic assay<sup>19</sup> which detected as little as 10 pg of DL-octopamine. The identity of the assay products was checked by several chromatographic and electrophoretic techniques<sup>6</sup>. The mean amount of octopamine found per DUMETi soma ( $\pm$ s.e.m.) was  $0.10 \pm 0.02$  pmol. Control assays performed on pools of up to 37 fast extensor motoneurone cell bodies gave no detectable octopamine. The minimum concentration ratio between the two neuronal types is therefore 800:1. Octopamine was also detected in pieces of extensor nerve which contained the axon of DUMETi but was undetectable in pieces of the same nerve which on physiological evidence were known to contain only the axons of the slow extensor and common inhibitor motoneurons. Of the neurones innervating the extensor muscle, therefore, only DUMETi contains octopamine. Octopamine is about four times more concentrated in the axon of DUMETi than in its soma.

We were first alerted to the possibility that DUMETi is

**Fig. 2** A dorsal view of a locust metathoracic ganglion showing the medial group of cell bodies stained with the dye neutral red ( $0.01 \text{ mg ml}^{-1}$  in isotonic locust saline for 3 h at room temperature). The group contains two quite distinct size classes, large (L) and small (S) cells. The soma of the DUMETi neurone is a member of the group of large cells. The organisation of the group is variable, the soma of DUMETi can be found anywhere among the group of large cells. Compare ganglion outline with Fig. 1a. A, Anterior; P, posterior. Scale bar, 100  $\mu$ m.



**Fig. 3** a, The effect of stimulating DUMETi on extensor muscle tension generated by the slow extensor motoneurone. The time base is expanded below to show the individual twitches of the muscle (R4), the soma spikes (R1) and axon spikes (R2) of DUMETi. The slow motoneurone was stimulated at 10 Hz. Note the slow potentiation and long lasting effect of DUMETi on tension. b, The effect of stimulating DUMETi on EPSP amplitude (R5). DUMETi is stimulated antidromically (S1) at 20 Hz. At this frequency the soma spikes are unable to follow 1:1 and fail to initiate at all after the first few seconds of stimulation. A few soma spikes can be seen during the initial seconds of stimulation (R1); subsequently only the smaller axon spike is recorded in the soma. c, The effect of  $10^{-6}$  M DL-octopamine in saline on the EPSP (R5) and tension (R4) generated by stimulating the slow motoneurone (S2). d, The oscilloscope is triggered by the stimulus delivered to the slow motoneurone (S2). Each EPSP (R5) produces a response in the tension transducer (R4). A series of six triggered sweeps about 5 s apart starting at the arrival of octopamine are shown. They show a graded increase in the EPSP amplitude and a graded increase in the tension. e, The effect of  $10^{-6}$  M octopamine in saline on the amplitude of IPSP (R5) elicited by stimulating the common inhibitory motoneurone. On the left a graded decline in the IPSP amplitude following superfusion with octopamine and on the right there is a graded recovery of the IPSP amplitude when the octopamine solution is replaced with saline. The oscilloscope is triggered by the stimulus delivered to the common inhibitor axon (S4) and five sweeps about 5 s apart are superimposed.

involved in synaptic modulation by our observation that the tension generated by the slow extensor motoneurone was potentiated by DUMETi stimulation (Fig. 3a) (M.O. and P.D.E. in preparation). The only mechanical effect of stimulating DUMETi alone is a small decrease in basal tonus. Exogenously applied octopamine enhances the tension evoked in the extensor muscle by the slow motoneurone (Fig. 3c) and this effect is blocked by the  $\alpha$ -adrenergic blocking agent, phentolamine. The increase in tension ( $<30\%$ ) is accompanied by a facilitation of the EPSP evoked by the slow motoneurone (Fig. 3b-d). In contrast, the IPSP elicited by stimulating the common inhibitory motoneurone is depressed by low concentrations of octopamine (Fig. 3e). These effects have a prolonged time course of many seconds. We could detect no effect of octopamine on the response of the muscle to stimulating the fast motoneurone.

We do not yet know the site of action or the mechanism of the synaptic modulation by octopamine. Octopamine could act on the postsynaptic muscle membrane or on the presynaptic terminals of the slow extensor and common inhibitory motoneurons and several mechanisms could mediate the effects shown here. Our demonstration, however, that octopamine can enhance excitation and depress



inhibitory neuromuscular synaptic transmission supports the growing evidence that amines have widespread modulatory roles<sup>7,20-22</sup>.

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1. Axelrod, J. & Saavedra, J. M. *Nature* **265**, 501-504 (1977).
2. Molinoff, P. B. & Axelrod, J. *J. Neurochem.* **19**, 157-163 (1972).
3. Robertson, H. A. & Juorio, A. V. *Int. Rev. Neurobiol.* **19**, 173-224 (1976).
4. Saavedra, J. M., Brownstein, M. J., Carpenter, D. O. & Axelrod, J. *Science* **185**, 364-365 (1974).
5. Wallace, B. G., Talamo, B. R., Evans, P. D. & Kravitz, E. A. *Brain Res.* **74**, 349-355 (1974).
6. Evans, P. D., Kravitz, E. A., Talamo, B. R. & Wallace, B. G. *J. Physiol., Lond.* **262**, 51-70 (1976).
7. Evans, P. D., Talamo, B. R. & Kravitz, E. A. *Brain Res.* **90**, 340-347 (1975).
8. Evans, P. D., Kravitz, E. A. & Talamo, B. R. *J. Physiol., Lond.* **262**, 71-89 (1976).
9. Carpenter, D. O. & Gaubatz, G. L. *Nature* **252**, 483-485 (1974).
10. Nathanson, J. A. & Greengard, P. *Science* **180**, 308-310 (1973).
11. Usherwood, P. N. R. & Grundfest, H. *J. Neurophysiol.* **28**, 497-518 (1965).
12. Pearson, K. G. & Bergman, S. J. *J. exp. Biol.* **50**, 445-471 (1969).
13. Hoyle, G. & Burrows, M. *J. Neurobiol.* **4**, 3-41 (1973).
14. Hoyle, G., Dagan, D., Moberly, B. & Colquhoun, W. *J. exp. Zool.* **187**, 159-165 (1974).
15. Plotnikova, S. I. *J. Evol. Biochem. Physiol.* **5**, 339-341 (1969).
16. Crossman, A. R., Kerkut, G. A., Pitman, R. M. & Walker, R. J. *Comp. Biochem. Physiol.* **40A**, 579-594 (1971).
17. Stuart, A. E., Hudspeth, A. J. & Hall, Z. W. *Cell Tissue Res.* **153**, 55-61 (1974).
18. Hoyle, G. & Barker, D. L. *J. exp. Zool.* **193**, 433-439 (1975).
19. Molinoff, P. B., Lansberg, L. & Axelrod, J. *J. Pharmac. exp. Ther.* **170**, 253-261 (1969).
20. Antelman, S. M. & Caggiula, A. R. *Science* **195**, 646-653 (1977).
21. Scabelli, H. C. & Mosnaim, A. D. *Am. J. Psychiat.* **131**, 695-699 (1974).
22. Weiss, K. R., Cohen, J. & Kupfermann, I. *Brain Res.* **99**, 381-386 (1975).

## Magnetic ferrofluids for preparation of magnetic polymers and their application in affinity chromatography

THE use of magnetic polymers as supports for biological molecules has created much interest<sup>1-5</sup>. The inherent advantages of such preparations are, in particular, the ease of recovery of these polymers by applying a magnetic field. Thus, when used as supports for immobilised enzymes, their easy retrieval from liquors containing colloids or undissolved solids should be of great practical value<sup>1-3</sup>. Magnetic polymers have recently been tested as an alternative to conventional radioimmunoassay technique, obviating the need for vertical rotation and for the time-consuming, multiple centrifugations required with conventional solid phase procedures<sup>1-5</sup>. In the established techniques for producing magnetic polymer particles, the attachment of the biomolecules had to follow the preparation of the specific magnetic material; furthermore most of these preparations have been non-porous, exhibiting rather poor capacity. We describe here a novel and general procedure using magnetic fluids which allows 'post-magnetisation' of polymers already substituted with biological molecules. The properties of such preparations have been tested primarily as affinity chromatography gels. These preparations showed unaltered biospecificity when applied in general ligand-affinity chromatography studies. The simplified separation possible due to the magnetic properties of the gels eliminates the usual centrifugation and column chromatography steps. The procedure of 'post-magnetisation' seems to be suitable for different polymer particles, both unsubstituted gels and those carrying immobilised ligands as affinity adsorbents or enzymes.

The basic new principle in the procedure described here is the use of magnetic fluids. These can be characterised as fluids consisting of ultramicroscopic particles (~100 Å) of oxides. These are stabilised by a coating, typically 25-Å thick, preventing their sticking together in a magnetic field. Random collisions (Brownian motion) with the carrier

liquid's molecules keep the particles in colloidal suspension and are additionally stabilised by physicochemical means. In the following, ferrofluids, that is, those containing ferrite particles, have been used and with either water or hydrocarbon as the carrier. They behave like true homogeneous fluids, yet are highly susceptible to magnetic fields.

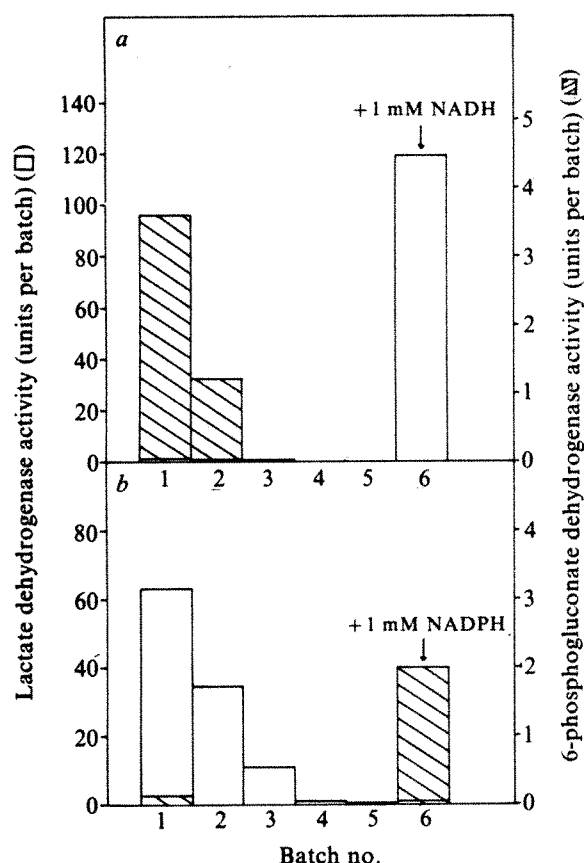
One gram of moist 5'-AMP- (or 2', 5'-ADP) -Sephacrose 4B (Pharmacia) which was previously swollen in 0.1 M sodium phosphate buffer pH 7.5, and washed with H<sub>2</sub>O was packed in a column. Subsequently 6.5 ml of ferrofluid (base, H<sub>2</sub>O; magnetic saturation, 200 G; trademark A05, from Ferrofluidics Corporation, Burlington, Mass.) were pumped through the column and cycled for 4 h at a flow rate of 50 ml h<sup>-1</sup> (usually at room temperature). After washing with 1 l of H<sub>2</sub>O, on glass filter, incubation overnight at 4 °C with 100 mg of bovine serum albumin in 0.1 M Tris-HCl buffer pH 7.6, 5 mM in EDTA and 1 mM 2-mercaptoethanol was carried out, followed by washing with 200 ml 1 M NaCl and 200 ml of the same buffer. The dark brown gel beads were then magnetic and ready for use (Some enzymes tested are slowly inactivated, for reasons at present not yet definitely established, unless albumin treatment is carried out.)

When applying ferrofluids with a hydrocarbon as a base, an alternative procedure also involving albumin treatment was applied. The hydrocarbon-based ferrofluid (hydrocarbon; trademark HO1, magnetic saturation: 200 G) was circulated through the bed containing beads previously treated with solutions of gradually changing composition going from water to acetone. After the ferrofluid treatment the beads were filtered on a glass filter and washed with acetone. This was followed by successive washing with different solvent mixtures in the sequence: acetone, acetone-cyclohexane, cyclohexane, cyclohexane-acetone, acetone, water and finally buffer. To further increase the magnetic properties of the latter preparations they were treated once more before the final acetone washing. No differences in the affinity behaviour of the two types of preparations were noticed.

Whether the ferrite particles simply adsorb to the gel or precipitate out in the interior of the beads remains to be established. (In this context it should be noted that entrapment of ferrofluid within the lattice of polyacrylamide can easily be accomplished.) In any case, the magnetic properties of the gel remain unchanged after extensive washing with buffer (even washing with, for example, ethanol or 40% ethylene glycol did not remove the ferrite particles) and repeated use in magnetic fields; they are strong enough to allow rapid sedimentation even when applying weak permanent magnets.

To establish the usefulness of the magnetic Sepharose beads for affinity chromatography, we had to establish whether the biospecificity of the immobilised ligands remained unchanged, and to investigate the capacity of the gels. To study the former aspect, beef heart lactate dehydrogenase was chosen as model enzyme and applied to magnetised 5'-AMP-Sepharose 4B. As expected it bound to the 5'-AMP-gel, known to be specific for NAD<sup>+</sup>-dependent enzymes, and could subsequently be eluted quantitatively with 1 mM NADH (Fig. 1a). In contrast the NADP<sup>+</sup>-dependent enzyme 6-phosphogluconate dehydrogenase, chosen as reference, showed no affinity for the gel, which is in accord with previous findings using non-magnetised preparations of this general ligand<sup>6</sup>. Conversely, lactate dehydrogenase would not bind to 2', 5'-ADP, known to be specific for NADP<sup>+</sup>-dependent enzymes, in contrast to 6-phosphogluconate dehydrogenase, which bound and could be subsequently eluted with 1 mM NADPH (Fig. 1b).

To further investigate the usefulness of the magnetic affinity material prepared, isozyme separation of horse liver alcohol dehydrogenase was attempted. On applying a



**Fig. 1** *a*, To 0.4 g of moist magnetic 5'-AMP-Sepharose 4B (ferrofluid  $H_2O$ ) were added 118 units (0.36 mg) of lactate dehydrogenase (from beef heart) or 4.5 units of phosphogluconate dehydrogenase (0.8 mg) from yeast in 2 ml of 0.1 M Tris-HCl, pH 7.6 containing 5 mM EDTA and 1 mM 2-mercaptoethanol. After incubation for 30 min on a rocking table (alternatively the gel could be kept suspended through a magnet kept at the outside of a rotating glass tube), the gel was settled in a beaker by applying at the outside a cylinder-formed permanent magnet with a pull of approximately 5 kg. After removal of the 'supernatant' from the gel (batch no. 1), the latter was washed 15 min in  $4 \times 10$  ml of the incubation buffer (batch nos 2-5). Elution of the affinity-bound enzyme was achieved by incubation for 15 min in 10 ml of 1 mM NADH in the buffer. All experimental manipulations were carried out at 4 °C and enzymes assayed as described earlier<sup>6</sup>; batch no. on the abscissa refers to the number of successive washings and elution carried out with one affinity gel preparation. *b*, To 0.4 g of moist magnetic 2',5'-ADP-Sepharose 4B (ferrofluid, hydrocarbon) were added, as above, either lactate dehydrogenase or 6-phosphogluconate dehydrogenase. The experimental procedures were as for (a) except for the use of 1 mM NADPH.

mixture of the three isozyme forms EE, ES, and SS to magnetised 5'-AMP-Sepharose 4B, we found that they would adsorb to the gel. Afterwards a pulse of  $NAD^+$  plus cholic acid eluted primarily the steroid active isozyme SS. With a subsequent application of NADH, the EE and ES forms were eluted, which is in analogy to previous findings<sup>7</sup>. It was found that the ratio of ethanol to steroid (5 $\beta$ -dihydrotestosterone) activity in the fractions eluted with  $NAD^+$  plus cholic acid showed, after only one batch treatment, the low value of 1.3; this compares with 1.1 found for completely pure SS isozyme. (The applied mixture had a ratio of 7.) Clearly the elution technique used previously, which leads to purification of the SS isozyme, is applicable also to the magnetic polymer particles<sup>8</sup>.

Finally, a magnetised 5'-AMP-Sepharose 4B preparation was used for the purification of horse liver alcohol dehydrogenase from crude extracts<sup>7</sup>. As shown in Table 1, alcohol dehydrogenase of high specific activity was obtained in one step. The enzyme was almost pure as judged from sodium dodecylsulphate-gel electrophoresis. The procedure com-

**Table 1** Purification of horse liver alcohol dehydrogenase by magnetic 'general ligand-affinity chromatography' in one step

Step	Specific activity (U mg <sup>-1</sup> )	Purification (n-fold)	Capacity (mg of protein per g moist gel)
Crude extract	0.08	1	
Magnetic 5'-AMP-Sepharose 4 B	1.70	21	0.7
5'-AMP-Sepharose 4B	1.98	25	1.5

A crude extract of horse liver was prepared as previously described<sup>7</sup> with a protein concentration of 46.4 mg ml<sup>-1</sup>. A large excess of protein (100-200 mg) in relation to 1 g of magnetic moist 5'-AMP (ferrofluid hydrocarbon)-Sepharose present in a beaker was applied. After incubation for 60 min on a rocking table, the gel was settled in a magnetic field and the 'supernatant' removed. Before elution of the affinity-bound enzyme, the gel was washed extensively with 0.1 M sodium phosphate buffer, pH 7.5 followed by a wash for 15 min in 0.1 mM  $NAD^+$  (4 ml) and the above buffer ( $3 \times 10$  ml). The enzyme was then eluted by addition to the gel of 3 ml of 0.1 mM  $NAD^+$  + 5 mM pyrazole in the above buffer (30 min, 4 °C). The lower set of values refers to a parallel run using identical amounts of non-magnetic affinity gel.

pared well with a blank run using non-magnetised 5'-AMP-Sepharose 4B, also in batch-fashion; only the capacity of the gel was diminished to about half. But its value of 0.7 mg of enzyme per g of moist gel is high when compared with some of the magnetic gels prepared in the beginning of this study following conventional approaches as entrapment of  $Fe_3O_4$  particles inside gels.

A further advantage of the use of magnetic polymers in affinity chromatography lies in the fact that it allows a rapid 'pick-up' of molecules from colloidal solutions or those containing cell debris. Such quick retrieval would be most difficult or impossible to accomplish by conventional purification methods. This advantage seems to be of particular value if a rapid isolation of labile enzymes or enzyme complexes after their liberation from the cell is required. It is likely that once the enzyme is affinity-adsorbed within the pores of a gel, it is more protected, for example, against proteolytic enzymes. To test the ease of enzyme isolation, the following model experiment was carried out: horse liver was quickly ground and immediately thereafter magnetic 5'-AMP-Sepharose beads were added to the homogenate. After incubation, the cell debris was decanted by keeping the gel particles at the bottom of the beaker through a permanent magnet placed at the outside. After several batch-washings, alcohol dehydrogenase was ready to be eluted in  $NAD^+$  plus pyrazole. Although the enzyme obtained showed lower specific activity (0.65 units mg<sup>-1</sup>) than that given in Table 1, it clearly demonstrates the potential of the procedure.

Thus the simple technique applied here for the 'post-magnetisation' of pre-formed polymers already substituted with ligands has been shown not to alter the affinity properties of the polymers tested. This technique has also been applied successfully to unsubstituted gels such as Sepharose 4B or Sepharose Cl 6B. In addition, magnetic enzyme-bearing polymers were prepared applying the procedure described. Using trypsin as model enzyme, magnetic Sepharose-trypsin preparations were obtained with equivalent specific activities relative to soluble enzyme, as found with normal Sepharose-coupled trypsin preparations. In a typical experiment 5 mg of trypsin (bovine pancreas, Sigma type III) were allowed to couple to 1 g of moist Sepharose 4B activated by the CNBr procedure<sup>9</sup>. After washing, the Sepharose-trypsin preparation was treated for 4 h at 4 °C with 6 ml of ferrofluid ( $H_2O$  as base) in analogous manner to the procedure given.

Alternatively, the Sepharose beads were first magnetised and then underwent CNBr activation and trypsin coupling.

The latter preparation showed higher specific activity (relative to soluble enzyme) compared with the former (30% compared with 20%) but was somewhat less magnetic.

It seems that the magnetisation procedure described here using magnetic fluids will be of general interest, since in addition these fluids are also relatively inexpensive. We have demonstrated here the usefulness of the procedure for the preparation of suitable magnetic-affinity chromatography systems and for the preparation of magnetic enzyme-bearing polymers, the latter with potential in the area of enzyme technology. One can envisage other areas in which similarly prepared magnetic biomolecule-polymer complexes may find application, for instance as components of immunoassay systems; similarly magnetic carrier systems for 'targeting of drugs' could be designed carrying drugs, enzymes or other molecules of therapeutic value, which through a magnetic field can be brought to the desired site of action. In contrast to previous methods of preparing magnetic particles whereby in order to obtain the required magnetic properties one is forced into a choice of particular polymer, magnetic particle or procedure (often at the expense of other desired properties as porosity), such considerations are not involved in the simple procedure given here.

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1. Munro, P. A., Dunnill, P. & Lilly, M. D. *Biotechnol. Bioengng* **19**, 101-124 (1977).
2. Horisberger, M. *Biotechnol. Bioengng* **18**, 1647-1651 (1976).
3. Chaplin, M. F. & Kennedy, J. F. *Carbohydr. Res.* **50**, 267-274 (1976).
4. Lynn, N. *et al. Clin. chim. Acta* **69**, 387-396 (1976).
5. Hersh, L. S. & Yaverbaum, S. *Clin. chim. Acta* **63**, 69-72 (1975).
6. Brodelius, P., Larsson, P. O. & Mosbach, K. *Eur. J. Biochem.* **47**, 81-89 (1974).
7. Andersson, L., Jörnval, H., Åkeson, Å. & Mosbach, K. *Biochim. Biophys. Acta* **364**, 1-8 (1974).
8. Andersson, L., Jörnval, H. & Mosbach, K. *Analyt. Biochem.* **69**, 401-409 (1975).
9. Porath, J. & Axen, R. *Methods Enzym.* **44**, 19-45 (1976).

## Differential labelling of $\alpha$ and $\beta$ -noradrenergic receptors in calf cerebellum membranes with $^3\text{H}$ -adrenaline

$\beta$ -NORADRENERGIC receptor detection with radiolabelled catecholamines has been hampered by binding of catecholamines to other sites<sup>1,2</sup>. Lefkowitz and Williams<sup>3</sup> described binding of a synthetic catecholamine  $^3\text{H}$ -hydroxybenzylisoprenaline to  $\beta$ -noradrenergic receptors on frog red blood cell membranes. Using procedures to prevent metabolism and nonspecific binding, we identified binding of  $^3\text{H}$ -adrenaline to  $\alpha$ -noradrenergic receptor sites in brain membranes<sup>4,5</sup>. We report here the labelling of  $\beta$ -noradrenergic receptors in membranes of the calf cerebellum with  $^3\text{H}$ -adrenaline.

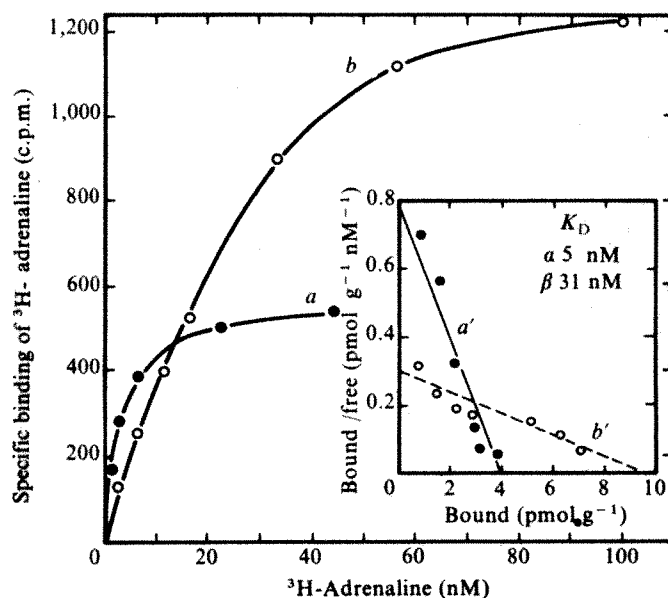
Homogenates of fresh or frozen calf cerebellar cortex were prepared as described previously<sup>4</sup>. Triplicate incubation tubes received 20  $\mu\text{l}$  of diluted  $^3\text{H}$ -adrenaline (DL-7,  $^3\text{H}$ (N)-epinephrine L-bitartrate, 11 Ci mmol<sup>-1</sup>, New England Nuclear) to give a final concentration of 10 nM  $^3\text{H}$ -adrenaline, 10  $\mu\text{l}$  of various concentrations of drugs, 20  $\mu\text{l}$  of a solution containing 50 mM pyrocatechol, 5 mM disodium EDTA and 0.5 mM dithiothreitol, and 0.94 ml of tissue suspension. In  $\beta$ -receptor binding experiments,  $\alpha$ -receptor binding was eliminated by the inclusion of 1.0  $\mu\text{M}$  phentolamine in incubation solutions. Conversely, in  $\alpha$ -receptor binding experiments, 1.0  $\mu\text{M}$  ( $\pm$ )-propranolol was included to abolish  $\beta$ -receptor binding of  $^3\text{H}$ -adrenaline.  $^3\text{H}$ -Adrenaline and all other drugs were made up in 0.1%

ascorbic acid. The tubes were incubated at 25 °C for 60 min, and then rapidly filtered under vacuum through Whatman GF/B filters presoaked in 1 mM pyrocatechol. The filters were rinsed with 16 ml of ice-cold 50 mM Tris-HCl buffer containing 1 mM pyrocatechol and 0.1% ascorbic acid, and were subsequently counted by liquid scintillation spectrometry in 10 ml Formula 947 (New England Nuclear) at 37% efficiency.

Saturable or specific binding of  $^3\text{H}$ -adrenaline to  $\alpha$ -receptors was measured in the presence of 1.0  $\mu\text{M}$  ( $\pm$ )-propranolol, as the excess over blanks which included 1.0  $\mu\text{M}$  oxymetazoline. Saturable or specific  $\beta$ -receptor  $^3\text{H}$ -adrenaline binding was measured, in the presence of 1.0  $\mu\text{M}$  phentolamine, as the excess over blanks which included 1.0  $\mu\text{M}$  ( $\pm$ )-propranolol. Previous experiments have demonstrated that after incubation of  $^3\text{H}$ -adrenaline with calf brain membranes for 60 min at 25 °C in the conditions mentioned here, radioactivity eluted from the membrane pellet and radioactivity in the supernatant was essentially identical to cochromatographed stock  $^3\text{H}$ -adrenaline<sup>5</sup>.

In the presence of 1  $\mu\text{M}$  phentolamine, specific binding of  $^3\text{H}$ -adrenaline to calf cerebellar membranes was saturable (Fig. 1). Scatchard analysis revealed a single population of binding sites, with a dissociation constant ( $K_D$ ) of 31 nM. The specificity of  $^3\text{H}$ -adrenaline binding in the presence of 1  $\mu\text{M}$  phentolamine fulfilled characteristics expected of  $\beta$ -noradrenergic receptors. Of the principal catecholamines, ( $-$ )-isoprenaline was most potent in competing for binding ( $K_i=1.9$  nM), exceeding the potencies of ( $-$ )-adrenaline and ( $-$ )-noradrenaline by ten- and 100-fold respectively (Table 1). The 10-fold greater potency of adrenaline than of noradrenaline indicated that in calf cerebellar membranes,  $^3\text{H}$ -adrenaline binding was associated with  $\beta_2$  receptors. Binding of  $^3\text{H}$ -adrenaline in the presence of 1  $\mu\text{M}$  phentolamine was stereospecific,

**Fig. 1** Specific  $^3\text{H}$ -adrenaline binding to  $\alpha$ - and  $\beta$ -receptors as a function of increasing concentrations of  $^3\text{H}$ -adrenaline. Calf cerebellar homogenates (20 mg original wet weight of tissue) incubated for 60 min at 25 °C as described in the text, with various concentrations of  $^3\text{H}$ -adrenaline. *a*, Binding to  $\alpha$ -receptors, measured in the presence of 1.0  $\mu\text{M}$  ( $\pm$ )-propranolol. Specific binding was that displaceable by 1.0  $\mu\text{M}$  oxymetazoline. *b*, Binding to  $\beta$ -receptors, measured in the presence of 1.0  $\mu\text{M}$  phentolamine. Specific binding was that displaceable by 1.0  $\mu\text{M}$  ( $\pm$ )-propranolol. In each case, specific binding was defined as the difference between total and nonspecific. Points shown are those obtained in a single experiment, performed in triplicate, which was replicated three times. *Inset*. Scatchard plots, showing  $K_D$  values of 5 nM at  $\alpha$ -receptors, and 31 nM at  $\beta$ -receptors. Specific binding of  $^3\text{H}$ -adrenaline to  $\alpha$ -receptors at a concentration of 70 nM was not significantly different from that obtained at 40 nM  $^3\text{H}$ -adrenaline, and is not shown in the concentration-binding curve, but is included in the Scatchard plot.





with (–)-catecholamines being 30–150 times more potent than the corresponding (+)-catecholamines. Affinities of other  $\beta$ -agonists paralleled their known pharmacological potencies and affinities for sites labelled by radioactive  $\beta$ -antagonists<sup>6</sup>.  $\beta$ -Noradrenergic antagonists competed potently and stereospecifically, with (–)-propranolol and (–)-alprenolol displaying apparent  $K_i$  values of 2–3 nM. Dichloroisoprenaline, (–)-practolol and butoxamine, pharmacologically weaker  $\beta$ -antagonists<sup>7</sup> were comparably weaker in competing for binding of <sup>3</sup>H-adrenaline. At concentrations as high as 10  $\mu$ M, drugs active at  $\alpha$ -noradrenergic receptor sites failed to influence the binding of <sup>3</sup>H-adrenaline, in the presence of 1  $\mu$ M phentolamine.

In the presence of 1  $\mu$ M ( $\pm$ )-propranolol, <sup>3</sup>H-adrenaline seemed to bind selectively to  $\alpha$ -receptor sites on calf cerebellar membranes, as reported earlier with calf cerebral cortex<sup>4,5</sup> (Fig. 1, Table 1). In these conditions, the specific binding of <sup>3</sup>H-adrenaline was saturable with maximal binding occurring by 20 nM. Scatchard analysis indicated a single population of binding sites, with a  $K_D$  value of 5 nM, similar to that found in the cerebral cortex. Thus <sup>3</sup>H-adrenaline displayed about six times greater affinity for  $\alpha$ - than for  $\beta$ -receptors. The substrate specificity of <sup>3</sup>H-adrenaline binding in the presence of propranolol was as expected at  $\alpha$ -receptor sites, with (–)-noradrenaline almost 100 times more potent than (–)-isoprenaline. The most potent catecholamine at apparent  $\alpha$ -sites was (–)-adrenaline, with an affinity three times greater than (–)-noradrenaline. The  $\alpha$ -agonist clonidine, the partial agonists ergotamine and piperoxan, and the  $\alpha$ -antagonists phentolamine and WB-4101 were potent competitors for the binding of <sup>3</sup>H-adrenaline in the presence of propranolol, with apparent  $K_i$  values in the 1–20-nM range. In general, the affinities of  $\alpha$ -receptor agonists and antagonists in competing for <sup>3</sup>H-adrenaline binding in the cerebellum in the presence of propranolol closely matched their affinities in competing for  $\alpha$ -receptor binding by <sup>3</sup>H-adrenaline, <sup>3</sup>H-noradrenaline and <sup>3</sup>H-clonidine in calf cerebral cortex<sup>4,5</sup> and rat brain<sup>8,9</sup>. Specificity of the binding of <sup>3</sup>H-adrenaline to  $\alpha$ -receptors in the presence of propranolol was supported by the very weak influence of  $\beta$ -agonists and  $\beta$ -antagonists on this binding (Table 1).

Kinetic studies were carried out to establish that the binding of <sup>3</sup>H-adrenaline to both  $\alpha$ - and  $\beta$ -receptor components was reversible. Specific binding in the presence of propranolol showed the same characteristics of association and dissociation at 25 °C as in the calf cerebral cortex<sup>5</sup>. Dissociation was biphasic, with a  $t_{1/2}$  of about 1.5 min, slightly faster than in the cortex. Seventy to eighty per cent of specific binding was dissociated after 10 min. Association of <sup>3</sup>H-adrenaline to  $\beta$ -receptors (in the presence of phentolamine) was more rapid than to  $\alpha$ -receptors; equilibrium binding, attained in 15 min, remained stable for 2 h. A standard incubation time of 60 min was chosen to parallel conditions for measuring binding to  $\alpha$ -receptors. Dissociation caused by adding a large excess of propranolol was extremely rapid at 25 °C, with a  $t_{1/2}$  of about 20 s, and complete dissociation after 2 min.

In preliminary experiments, we failed to demonstrate binding of <sup>3</sup>H-adrenaline to  $\beta$ -receptor sites in several rat brain regions, or in non-cerebellar areas of the calf brain. The ability to label  $\beta$ -receptors with <sup>3</sup>H-adrenaline in calf cerebellum may be related to the greater density of  $\beta$ -noradrenergic receptors in this tissue detected by the binding of the  $\beta$ -antagonists, <sup>3</sup>H-dihydroalprenolol and <sup>125</sup>I-hydroxybenzylpindolol (ref. 10 and our unpublished work), and to the observation that  $\beta$ -receptor sites in calf cerebellum are of the  $\beta_2$  type, in which adrenaline is about 10 times more potent than noradrenaline.

Binding studies with a variety of neurotransmitter receptors in the brain have revealed distinct differences between interactions of agonists and antagonists<sup>8,11–15</sup>. Most studies of  $\beta$ -receptors in various tissues have used only a radiolabelled antagonist. Evaluation of important questions of receptor functioning, such as desensitisation, requires studies of the

**Table 1** Inhibition of <sup>3</sup>H-adrenaline binding to  $\alpha$ - and  $\beta$ -noradrenergic receptors in calf cerebellum

Drug	$K_i$ at $\alpha$ -receptor (nM)	$K_i$ at $\beta$ -receptor (nM)
<b>Catecholamines</b>		
(–)-Adrenaline	2.4	21
(+)-Adrenaline		580
(–)-Noradrenaline	8	220
(+)-Noradrenaline		25,000
(–)-Isoprenaline	700	1.9
(+)-Isoprenaline		280
<b><math>\alpha</math>-Agonists</b>		
Clonidine	1.7	> 10,000
Oxymetazoline		> 10,000
<b><math>\alpha</math>-Partial agonists</b>		
Ergotamine	6	> 10,000
Piperoxan	13	
<b><math>\alpha</math>-Antagonists</b>		
Phentolamine	1.3	> 10,000
WB-4101	21	
<b><math>\beta</math>-Agonists</b>		
Cc 25 (Desmethyl hydroxybenzylisoprenaline)		4
MJ 9184-1 (3-Methanesulphonamido-N-benzylisoprenaline)		6
Cc 34 (Hydroxybenzylisoprenaline)		25
Salbutamol	> 100,000	
Terbutaline	> 100,000	1,500
<b><math>\beta</math>-Antagonists</b>		
(–)-Propranolol	23,000	2.3
(+)-Propranolol	8,000	60
(–)-Alprenolol	13,000	2.4
(+)-Alprenolol	7,000	23
Dichloroisoprenaline	20,000	730
(–)-Practolol	> 100,000	7,300
Butoxamine	> 100,000	12,000

Calf cerebellar cortex homogenates were incubated with 10 nM <sup>3</sup>H-adrenaline for 60 min at 25 °C, in the presence of 1.0  $\mu$ M ( $\pm$ )-propranolol for  $\alpha$ -receptor binding, or 1.0  $\mu$ M phentolamine for  $\beta$ -receptor binding, together with 3–6 concentrations of unlabelled drugs, using the standard assay conditions as described in the text.  $IC_{50}$  values were determined by log-probit analysis, and apparent  $K_i$  values calculated from the equation  $K_i = IC_{50}/(1 + [^3H\text{-adrenaline}]/K_D)$ . The  $K_D$  values used were 5 nM for  $\alpha$ -receptor binding, and 31 nM for  $\beta$ -receptor binding. Values given are means from 2 to 4 experiments, each conducted in triplicate, which varied less than 20%.

direct binding of radiolabelled agonists. It is also desirable to label receptors with naturally-occurring transmitters or transmitter analogues. Accordingly, labelling  $\beta$ -receptors with <sup>3</sup>H-adrenaline may facilitate an evaluation of receptor properties. Further studies now in progress show that in calf cerebellum the affinities of agonists are about 10-fold higher at sites labelled by <sup>3</sup>H-adrenaline than at sites labelled by <sup>3</sup>H-dihydroalprenolol, with the converse applying to antagonist affinities. These data suggest that a “two-state” model of receptor function may be applicable to the  $\beta$ -noradrenergic receptor as well as to the  $\alpha$ -noradrenergic and other receptors in the central nervous system<sup>6,8</sup>, although the differences between receptor interactions of agonists and antagonists are less marked at  $\beta$ -receptors than at other transmitter receptors. The possibility of labelling both  $\alpha$ - and  $\beta$ -receptors in the same tissue with the same <sup>3</sup>H-ligand may prove valuable in attempts to differentiate the properties of these two types of noradrenergic receptors.

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1. Cuatrecasas, P., Tell, G. P. E., Sica V., Parikh, I. & Chang, K. J. *Nature* 247, 92-97 (1974).
2. Bar H. P. in *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (ed. Paton, D. M.) 247-257 (Raven, New York, 1976).
3. Lefkowitz, R. J. & Williams, L. T. *Proc. natn. Acad. Sci. U.S.A.* 74, 515-519 (1977).
4. U'Prichard, D. C. & Snyder, S. H. *Life Sci.* 20, 527-534 (1977).
5. U'Prichard, D. C. & Snyder, S. H. *J. biol. Chem.* (in the press).
6. Mukerjee, C., Caron, M. G., Mullikin, D. & Lefkowitz, R. J. *Molec. Pharmac.* 12, 16-31 (1976).
7. Mukerjee, C., Caron, M. G., Coverstone, M. & Lefkowitz, R. J. *J. biol. Chem.* 250, 4869-4876 (1975).
8. U'Prichard, D. C., Greenberg, D. A. & Snyder, S. H. *Molec. Pharmac.* 13, 454-473.
9. Greenberg, D. A. & Snyder, S. H. *Life Sci.* 20, 927-932 (1977).
10. Bylund, D. B., Charness, M. E. & Snyder, S. H. *J. Pharmac. exp. Ther.* 201, 644-653 (1977).
11. Bennett, J. P., Jr & Snyder, S. H. *Molec. Pharmac.* 12, 373-389 (1976).
12. Burt, D. R., Creese, I. & Snyder, S. H. *Molec. Pharmac.* 12, 800-812 (1976).
13. Seeman, P., Lee, T., Chau-Wong, M., Tedesco, J. & Wong, K. *Proc. natn. Acad. Sci. U.S.A.* 73, 4354-4358 (1976).
14. Birdsall, N. J. M. & Hulme, E. C. *J. Neurochem.* 27, 7-16 (1976).
15. Pert, C. B. & Snyder, S. H. *Molec. Pharmac.* 10, 868-879 (1974).

## Purine and pyrimidine mononucleotides depolarise neurones of explanted amphibian sympathetic ganglia

ADENINE nucleotides and nucleosides have been proposed as neurotransmitters<sup>1-3</sup>, in part based on their ability to inhibit spike activity in several peripheral<sup>1,4,5</sup> and central<sup>2,5,6</sup> sites. Only one preliminary study<sup>2</sup> has utilised intracellular recording to determine mechanisms of action of the purines in neurones. Because of their large size and simplified input-output relationships, neurones of sympathetic ganglia are a favourable model for intracellular recording. Although several studies have shown effects of ATP on peripheral ganglia<sup>7,8</sup>, none utilised intracellular recording nor checked specificity of responses with other purine and pyrimidine

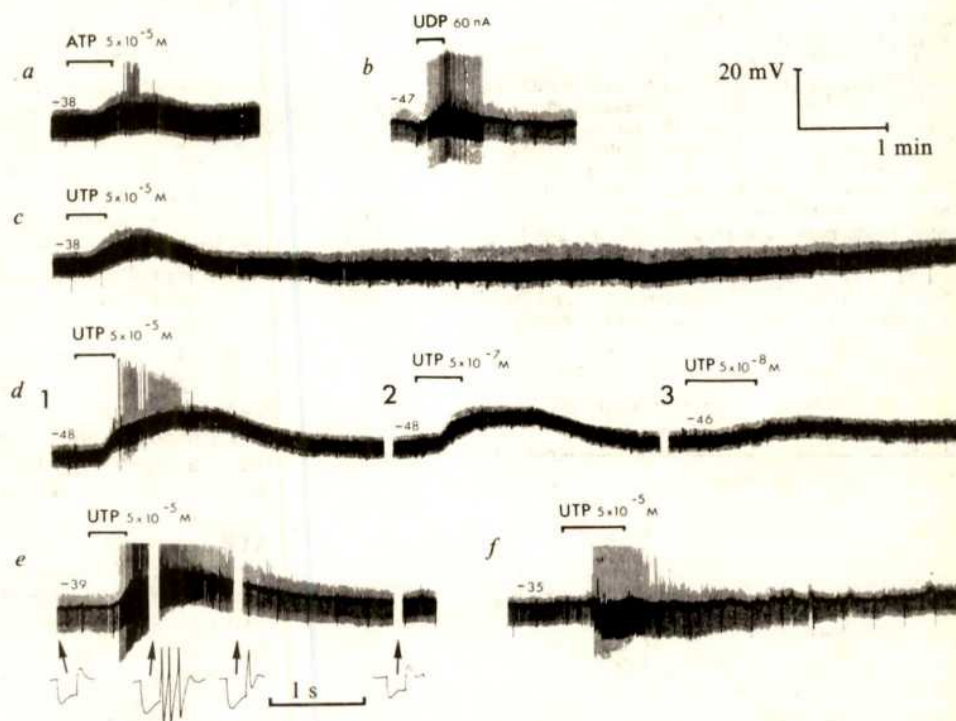
nucleic acid components. Therefore, we examined the effects of several purine and pyrimidine derivatives by intracellular recording of bullfrog sympathetic neurones in explant cultures. Our findings show pronounced depolarisations with the di- and triphosphoribo derivatives of several pyrimidine and purine bases, with the uridine nucleotides emerging as the most potent of all the compounds.

We studied 182 sympathetic neurones, cultured from adult bullfrogs and impaled with glass micropipettes (3 M KCl or potassium acetate; 8-40 MΩ impedance) under Nomarski optics. Intracellular stimulation was carried out by a bridge method<sup>10</sup> and current/voltage curves were constructed with a PDP-12 computer. The neurones studied ranged in size from 24 to 85 μm (long dimension) and displayed steady resting potentials of -35 to -58 mV with spike overshoots up to 30 mV or more. Drugs were applied by current-neutralised iontophoresis<sup>11</sup> or by rapid perfusion through a multiple valve system and a culture chamber with a volume of 0.3 ml.

Prolonged depolarisation was the predominant response to all the nucleic acid components studied, whether applied by perfusion or iontophoresis (Fig. 1a, b, d and e). Occasionally (14 of 98 cells) a long hyperpolarising component followed the depolarisations (Fig. 1c). In general, for any one purine or pyrimidine, the di- and tri-phosphonucleotides were equipotent as depolarising agents and more potent than their 5' monophosphonucleotides. The nucleosides guanosine, inosine and uridine and the heterocyclic bases guanine, adenine and uracyl were ineffective at concentrations up to 1-2.5 mM. Adenosine depolarised six of eleven neurones weakly at 2.5-5 mM.

Of all nucleotides studied, 5' uridine di- and triphosphate (UDP and UTP) were the most potent with thresholds for

**Fig. 1** Intracellular records of effects of mononucleotides on neurones cultured from the 8th-10th sympathetic ganglia of adult bullfrogs. Ganglia, cut into several pieces and placed on collagen coated coverslips, were cultured<sup>9</sup> for 1-3 months at 22 °C in the medium of McMahan and Kuffler<sup>14</sup>, also containing 0.3% methocel, streptomycin 100 μg ml<sup>-1</sup>, glutamine 0.8 mM, and usually, NGF 1 U ml<sup>-1</sup>. Heavy baselines in records show membrane potential (small numbers at beginning of each trace = mV). Light deflections below the baseline are responses to hyperpolarising constant current pulses of 0.05-1 nA and 200 ms duration, delivered through a Wheatstone bridge at 1 Hz. Examples of individual responses to current at higher polygraph speed below panel e. Pulses of 200 ms were used since this surpasses by 50-100 ms the initial unstable rectifying period typical of sympathetic neurones. Light lines above baseline are local depolarising responses to termination of current pulses (anodal break), which can trigger spikes. Heavy line descending below baseline every 20 s measures electrode resistance (1 MΩ/1 mV) by means of an RC circuit/ramp-input separate from the bridge circuit. a, Depolarising response to perfusion of ATP 5 × 10<sup>-5</sup> M, with production of anodal break spikes. b, Extracellular iontophoresis of UDP (0.2 M pH 6-7 in pipette) by anodal current of 60 nA depolarises another neurone with spiking. c, Another neurone: perfusion of UTP 5 × 10<sup>-5</sup> M causes depolarisation followed by a long hyperpolarisation; both phases accompanied by increases in input resistance, as assessed by size of hyperpolarising responses to constant current pulses, and increases in anodal break responses. d, Dose-related depolarisations of one neurone to UTP. e, Another cell: UTP 5 × 10<sup>-5</sup> M depolarises cell with intense spiking; high speed excerpts taken from this record at times indicated by arrows show multiple anodal-break spikes and an increase in input resistance during depolarisation; bar below record indicates time for high speed records. f, Same cell as e: UTP causes anodal-break spiking even while membrane potential is held at resting level by injection of continuous hyperpolarising current; calibration bar in upper right corner applies to all records except high speed excerpts of panel e.





depolarisation of about  $10^{-8}$  to  $5 \times 10^{-8}$  M; UTP  $5 \times 10^{-5}$  M produced depolarisations of 4–19 mV (mean = 10 mV;  $n=40$ ). Dose-related responses are shown in Fig. 1d. Thresholds for 5' ATP and 5' ADP were about  $10^{-6}$  to  $10^{-7}$  M; ATP  $5 \times 10^{-5}$  M depolarised an average of 5 mV ( $n=26$ ). Thymidine di- and triphosphate, guanosine di- and triphosphate and inosine di- and triphosphate were roughly equipotent with ATP. The uridine nucleotides seemed most potent also when applied by iontophoresis, producing depolarisations up to 16 mV; ATP and ADP evoked smaller depolarisations (up to 10 mV) with similar currents. Adenosine by iontophoresis was ineffective. Of all the nucleotides tested by perfusion, only 5' cytidine tri- and diphosphosphate were ineffective or only weakly active at  $5 \times 10^{-5}$  M. Pyrophosphate, 2'-deoxy-ATP, uridine diphosphoglucose, and D-ribose 5' phosphate were largely ineffective at concentrations ( $5 \times 10^{-5}$  to  $10^{-4}$  M) which were maximally effective for the nucleotides.

Both the depolarisations and the occasional ensuing hyperpolarisations were generally accompanied by an increase (47 out of 65 cells) or no measurable change in input resistance, as determined either by measuring responses to hyperpolarising constant current pulses (Fig. 1e), or by comparing current/voltage curves (Fig. 2). Only five cells showed resistance decreases. Preliminary tests of voltage dependence showed that the size of the nucleotide depolarisation was inversely related to membrane potential (two cells) or was not affected (two cells) by changes in membrane potential from -70 to -6 mV.

Changes in spike generation (produced by intracellular stimulation) also occurred during the nucleotide responses. Most commonly, thresholds for spiking decreased (Fig. 1a, b, d, e and f) during the initial phases of depolarisation (40 out of 69 cells), although occasionally thresholds

increased during the later phases. A pure increase in spike threshold occurred in 10 of the 69 cells. The nucleotide-evoked increases in resistance and spiking are not dependent on the depolarisations, since they are not diminished by holding the membrane potential at resting level with current during perfusion of the mononucleotide (Fig. 1f). Further, depolarising the membrane by current to the same level as evoked by the nucleotide had no equivalent effect on spiking and resistance.

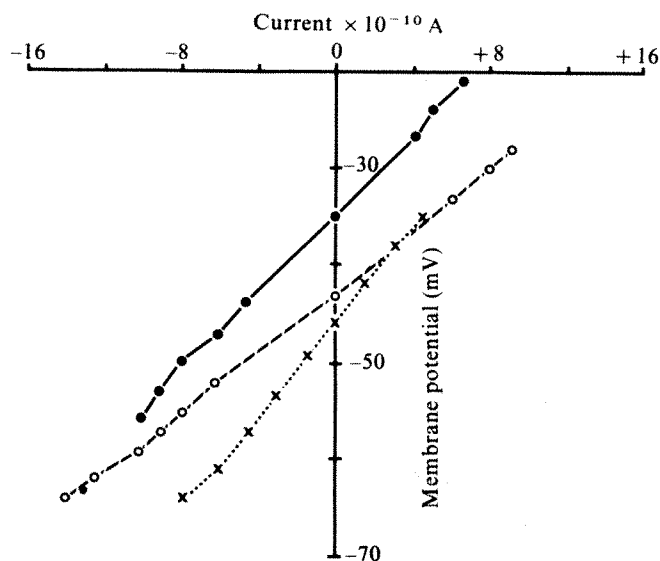
The mononucleotides act like the muscarinic cholinergic agent muscarine, which also depolarises cultured sympathetic neurones with increases in input resistance (G.R.S., D.L.G., A.L.P., and D.S.F. unpublished). The possibility that the nucleotides act by releasing endogenous acetylcholine, however, seems unlikely, since total blockade of the muscarine response by high concentrations of atropine ( $3 \times 10^{-6}$  to  $1.3 \times 10^{-5}$  M) has no effect on the responses to the uridine or adenine mononucleotides (six out of six cells). Perfusion with nicotine  $2.5$ – $5 \times 10^{-3}$  M (eight cells), (+)-tubocurarine 1 mM (four cells), or high  $Mg^{2+}$  (10–20 mM), low  $Ca^{2+}$  (0.5–1 mM) concentrations (six cells) also has little effect on the nucleotide responses. The fact that local application of UTP, UDP, ATP and ADP by iontophoresis markedly depolarised neurones, also argues for a direct effect of the nucleotides.

In preliminary studies, neither physostigmine ( $5 \times 10^{-5}$  M), hexamethonium (0.5–2.5 mM), phentolamine ( $10^{-4}$  M) nor sotatol ( $1.5 \times 10^{-4}$  M) altered responses to the nucleotides. Only quinidine ( $5 \times 10^{-4}$ ) blocked the UTP or ATP responses. This antagonism is, however, relatively unspecific, since the effects of muscarine are also blocked. The nucleotide receptor is not equivalent to the adenosine receptor of brain<sup>5,12</sup>, since in our preparation methyl xanthines (2.5 mM) do not antagonise, but in fact occasionally potentiate responses to the mononucleotides. This might indicate mediation of the response by intracellular cyclic nucleotides. Perfusion of high concentrations of either cyclic AMP ( $10^{-3}$ – $10^{-2}$  M), cyclic GMP ( $5 \times 10^{-3}$  M) or cyclic UMP ( $10^{-3}$  M), however, had only weak and variable effects on membrane potential, perhaps because of poor cell penetrability by the cyclic nucleotides. Finally, calcium chelation would not seem to be a factor in the nucleotide responses since such low effective concentrations of the nucleotides ( $10^{-7}$ – $10^{-8}$  M) are not likely to stoichiometrically remove significant  $Ca^{2+}$  from the Ringer's solution (containing 2 mM  $Ca^{2+}$ ), and since  $Ca^{2+}$  removal does not reduce the responses.

The potent responses to several of the mononucleotides might indicate their function as neurotransmitters in so-called 'purinergic' nerves or as 'co-transmitters' which might be released with acetylcholine or noradrenaline from autonomic nerves. In our preparation, the generalised effectiveness of purine and pyrimidine mononucleotides suggests that the 'purinergic' label may be too restrictive, especially since the uridine nucleotides are the most potent. In the *Torpedo* electric organ cholinergic stimulation releases ATP postjunctionally<sup>13</sup>. Our results suggest that such release could modulate or even mediate the response to a neurotransmitter. Indeed, responses to the nucleotides bear a strong resemblance to the ganglionic late slow excitatory postsynaptic potential, which is also atropine resistant. Preliminary results show that iontophoresis of UDP and UTP increases firing rates of several types of rat central neurones *in situ* (G.R.S. unpublished). Taken with the present finding of a potent depolarising action of uridine nucleotides in cultured sympathetic neurones, these results suggest that the uridine nucleotides warrant close scrutiny for their possible involvement in neurotransmission.

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**Fig. 2** Changes in current/voltage relationships of cultured sympathetic neurone in response to ADP. Curves were generated on-line by PDP-12 computer (DEC) using a modified ADTAPE program and FOCAL programs which select and average five consecutive digital points in equivalent control and pulse periods (200 ms duration) for current and voltage. During the current pulse, points were only sampled after the membrane potential had reached a steady level, generally 120–150 ms after pulse initiation (see high speed pulse records of Fig. 1e); care was taken to avoid selection of points where spikes occurred. ○, Control period; ●, peak of depolarisation to ADP  $10^{-4}$  M; X, peak of subsequent hyperpolarisation to ADP (see text). Increase in slopes of the latter two lines indicate increase in input resistance during both the 8 mV depolarisation and the 2.5 mV hyperpolarisation to ADP. Control input resistance = 16 MΩ.



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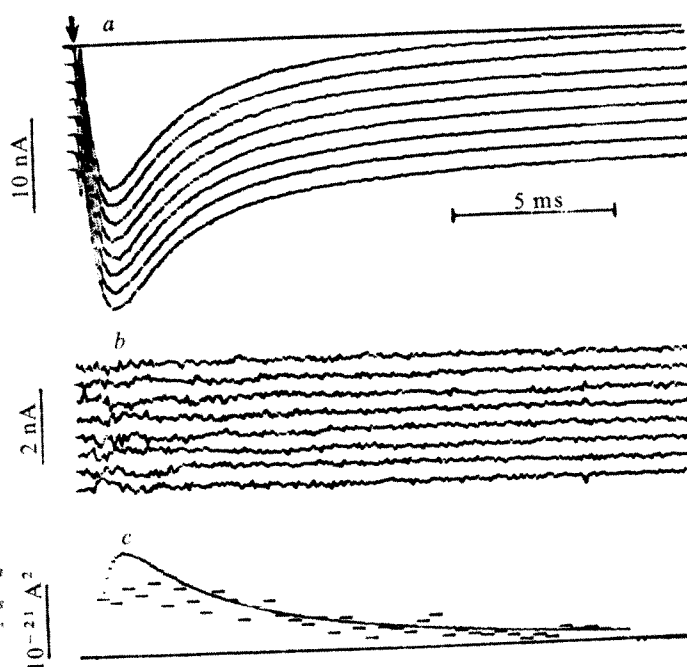
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1. Burnstock, G. *Pharmac. Rev.* **24**, 509–581 (1972).
2. Phillis, J. W. & Edstrom, J. P. *Life Sci.* **19**, 1041–1054 (1976).
3. Pull, I. & McIlwain, H. *Biochem. J.* **130**, 975–981 (1972).
4. Tomita, T. & Watanabe, H. *J. Physiol., Lond.* **231**, 167–177 (1973).
5. Siggins, G. R., Hoffer, B. J., Bloom, F. E. & Ungerstedt, U. in *The Basal Ganglia* (ed. Yahr, M.) 227–248 (Raven, New York, 1976).
6. Bloom, F. E., Siggins, G. R., Hoffer, B. J., Segal, M. & Oliver, A. P. in *Advances in Cyclic Nucleotide Research* (edit. Drummond, G., Greengard, P. & Robison, G. A.) 603–618 (Raven, New York, 1975).
7. Feldberg, W. & Hebb, C. J. *J. Physiol., Lond.* **107**, 210–221 (1948).
8. Theobald, R. J. & deGroat, W. C. *Fedn Proc.* **36**, 290 (1977).
9. Padjen, A. L., Siggins, G. R. & Forman, D. S. *Neurosci. Abs.* **1**, 813 (1975).
10. Araki, T. & Otani, T. *J. Neurophysiol.* **18**, 472–485 (1955).
11. Salmoiraghi, G. & Weight, F. *Anesthesiology* **28**, 54–64 (1967).
12. Sattin, A. & Rall, T. W. *Molec. Pharmacol.* **6**, 13–23 (1970).
13. Israel, M., Lesbats, B., Mennier, F. M. & Stinnakre, J. *Proc. R. Soc. Lond. B* **193**, 461–468 (1976).
14. McMahan, U. J. & Kuffler, S. W. *Proc. R. Soc. Lond. B* **177**, 485 (1971).

## Sodium channels in nerve apparently have two conductance states

THE inward sodium current underlying the nerve action potential is carried by discrete Na channels<sup>1</sup> in the membrane which are gated in response to the membrane potential. A basic question in understanding the gating mechanism is whether the conductance of the channels has only two states, open and closed, or whether there are more states, with the channel conductance progressing from one level to another to give the variable membrane conductance observed in voltage-clamp experiments. The ensemble fluctuation measurements reported here are consistent with the simple interpretation that Na channels have only two principal conductance states, with conductances of 0 and  $7.7 \pm 1$  pS (near zero membrane potential), and number approximately  $5 \times 10^4$  in the node of Ranvier of a 14  $\mu$ m diameter frog myelinated nerve fibre. Like stationary fluctuation analysis<sup>2</sup>, the experimental method described here can yield information about the size and kinetics of the single gating events in ionic channels, but is also applicable to non-stationary processes such as membrane conductances that activate or inactivate with time. I report here measurements of the mean Na current and the variance at various times after the start of a depolarising pulse, using an ensemble of successively evoked current transients.

The preparation used was *Rana temporaria*, node of Ranvier; this is useful for fluctuation experiments because a stable voltage clamp with low background noise is readily obtained and because the number of Na channels is in a convenient range ( $\lesssim 10^5$ ). Experiments were carried out at 2 °C to maximise the ratio of current fluctuations to thermal noise amplitude, and the voltage clamp electronics included a provision for measuring the membrane capacitance and the passive resistances of the intact preparation, allowing the thermal noise background to be estimated reliably. Potassium currents were blocked with internal Cs and external tetraethylammonium ions, and linear leak subtraction was used. A set of 64–160 Na current transients was elicited by repetitively depolarising the membrane at intervals of 300–500 ms. From groups of eight successive current records,



**Fig. 1** Ensemble variance calculation. *a*, Eight current records aligned with the start of the depolarising pulse (arrow). *b*, Residual fluctuations from each record after subtracting the mean of eight. *c*, Ensemble variance estimated from 72 records by the following process: the sum of the squares of the fluctuations was calculated for each time point; the resulting set of squared deviations was scaled and averaged with eight others; finally, the estimated background was subtracted. Each bar represents the mean of the resulting values at four adjacent time points. The smooth curve is the mean current record, scaled by a factor of  $-0.3$  pA. Node 13. Bathing Ringer solution contained 20 mM TEA-Cl; cut internodes were in 110 mM CsCl, 10 mM NaCl. Current records low-pass filtered at 5 kHz (4 pole, Gaussian response) and digitised (12 bits, 256 samples per record) at 100  $\mu$ s intervals; aperture time jitter was  $< 50$  ns. The thermal noise background was calculated (equations 11–16 of ref. 6) for each current sample, using the measured values for the internode and seal resistances and making use of the instantaneous *I-V* relation in estimating the membrane slope conductance. The peak value of the background component of the variance in *c* was  $7 \times 10^{-22} A^2$ .

mean and variance records were calculated point-by-point using a procedure similar to conventional signal averaging (Fig. 1). Several of these variance records were in turn averaged, and the conductance-dependent contribution from thermal and instrumental noise was calculated and subtracted, to yield an ensemble variance record. Figure 1c shows that the ensemble variance is proportional to the mean current at low conductance levels, but seems to saturate during the phase of high membrane conductance.

The contribution to the variance from processes other than channel gating was examined in several ways. First, the variance measured at  $V_0$ , the reversal potential for Na current, was found to be accounted for (within an uncertainty of  $2 \times 10^{-22} A^2$ ) by the calculated thermal noise background. Secondly, long term changes in the amplitude of Na current were monitored and were found to be  $< 2\%$  after 160 depolarisations in the experiments reported here. Because the mean and variance were calculated from small groups of successive records, linear drifts of that magnitude would not contribute significantly to the variance. Finally, the functional relationship between the variance and mean current values provides a clue to the source of the fluctuations. Any process which perturbs the properties of all the channels in the membrane coherently (for example, temperature or voltage fluctuations) would probably result in current fluctuations with variance increasing with the square of the mean current. Fluctuations in the transport of ions through



open channels would also be expected to give a monotonically increasing variance with increasing membrane conductance. Instead, the variance saturates and even decreases at high membrane conductance (Fig. 2), suggesting that neither of these processes is the predominant source of the fluctuations.

If the fluctuations were entirely due to Na channel gating, they could be interpreted on the basis of a simple open-closed scheme for the conductance of each channel. Assuming that the Na current is carried by a homogeneous population of  $N$  channels which are gated in a statistically independent fashion, the mean current  $I$  and the variance  $\sigma_i^2$  would be given by<sup>3</sup>

$$I = Npi$$

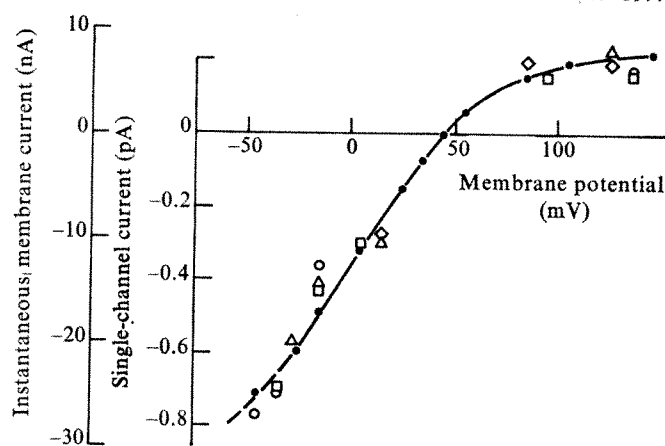
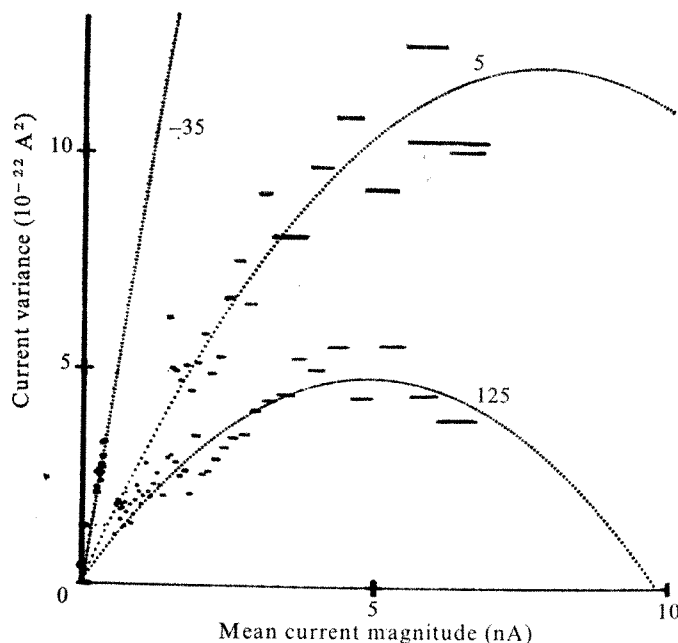
$$\sigma_i^2 = Np(1-p)i^2$$

$$i = \gamma(V - V_0)$$

where  $i$  is the current carried by a single channel,  $\gamma$  is the single-channel conductance,  $V$  is the membrane potential and  $V_0$  the reversal potential. The probability that a channel is in its open state is given by  $p$ , which is a function of both membrane potential and time. This theory assumes two conductance states but makes no assumption about the kinetics or voltage dependence of the gating process. A more specific theory based on the Hodgkin-Huxley equations would have  $p = m^3h$ .

The predictions of the two-state theory were tested in three ways. First, the relationship between  $I$  and  $\sigma_i^2$  was observed as  $p$  changes with time during each depolarisation. Plots of these quantities (Fig. 2) were fitted with the predicted quadratic function, and data obtained with different depolarisations of the same node could be fitted with the same value of  $N$ . Second, the voltage dependence of the single-channel current  $i$  was compared with the 'instantaneous' current-voltage relation of the membrane as determined by a voltage-jump experiment (Fig. 3). The values of  $i$  (estimated by curve fitting as in Fig. 2) represent either the single-channel current of the two-state

**Fig. 2** Variance plotted against the absolute value of mean current, with the time after the start of each depolarising pulse as the independent parameter. Points farthest from the origin correspond to the peak Na conductance. Depolarisations to  $-35$  and  $5$  mV (node 14) and  $125$  mV (node 8), all with  $-105$  mV,  $50$  ms prepulse from holding potential of  $-80$  mV. Theoretical curves drawn with  $N = 5 \times 10^4$ ;  $i = -0.7$ ,  $-0.31$ , and  $0.2$  pA, respectively.



**Fig. 3** Comparison of single channel currents and instantaneous  $I-V$  relation. Single channel currents from nodes  $\diamond$ , 8;  $\circ$ , 12;  $\triangle$ , 13;  $\square$ , 14. Curve follows instantaneous current values ( $\bullet$ ) from node 8, measured at the potential indicated after a 2 ms prepulse to  $V_0 = 44$  mV.

scheme or a weighted average of the various current levels in a multiple state scheme<sup>2</sup>. The similarity in voltage dependence argues against multiple state schemes in which the distribution among states of different conductance is voltage dependent.

Third, values of  $\gamma$  were estimated under conditions which modify the peak Na conductance of the membrane. Pre-pulses in the range  $-105$  to  $-55$  mV changed the peak conductance by a factor of 4 but caused no significant change in  $\gamma$ . Treatment with 8 nM tetrodotoxin reduced the currents by a factor of 5 but also left  $\gamma$  unchanged. Changing the Na concentration or the pH of the solution bathing the node changed  $\gamma$  from the control value of  $7.7 \pm 1$  pS measured at 0 mV in 90 mM Na to  $4.1 \pm 1.2$  pS in 55 mM Na and to  $2.5 \pm 1$  pS at pH = 5.2. Hydrogen ions are thought to alter the ion transport through open Na channels<sup>4,5</sup>.

Although this estimate of  $\gamma$  is similar to another measurement from nodes of Ranvier<sup>6</sup>, it may have been affected by the presence of transport noise or excessive filtering. An upper limit on the effect of transport noise can be estimated if all of the channels are assumed to be open at the peak conductance at 125 mV (Fig. 2). The variance in the peak current would then be entirely from transport fluctuations in open channels. Assuming the transport noise variance increases linearly with the Na conductance, this excess variance would cause my estimate of  $\gamma$  to be higher than the actual value by at most 40%. Alternatively,  $\gamma$  would have been underestimated if a step in the gating process has a relaxation time  $\leq 30$   $\mu$ s, for part of the resulting fluctuations would have been filtered. The estimates of the total number of channels  $N$  were in the range of  $3-6 \times 10^4$ , which corresponds to a Na channel density in the nodal membrane of roughly  $1,000 \mu\text{m}^{-2}$ . These values are tentative because the pool of channels that  $N$  represents may (because of ultraslow inactivation<sup>7</sup>, for example) be smaller than the total population in the membrane.

This work, like previous tests of the two-state theory<sup>6</sup>, has not ruled out all multiple conductance state schemes; there are theories with multiple conductance states which give predictions similar to those of the two-state theory. The simplest interpretation, however, is that Na channels have only two states of conductance.

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1. Hille, B. A. *Rev. Physiol.* **38**, 139-152 (1976).

2. Neher, E. & Stevens, C. F. *A. Rev. biophys. Bioengng* 6, 345–381 (1977).
3. Begenesich, T. & Stevens, C. F. *Biophys. J.* 15, 843–846 (1975).
4. Woodhull, A. J. *gen. Physiol.* 61, 687–708 (1973).
5. Drouin H. & Neumcke, B. *Pflügers Arch. ges. Physiol.* 351, 207–229 (1974).
6. Conti, F., Hille, B., Neumcke, B., Nonner, W. & Stämpfli, R. *J. Physiol., Lond.* 262, 699–728 (1977).
7. Fox, J. *Biochim. biophys. Acta* 426, 232–244 (1976).

## Modulation of electrical activity in *Aplysia* neurones by cholesterol

VARIOUS hormones and drugs induce long-lasting alterations of electrical activity in certain neurones from the marine snail *Aplysia californica*<sup>1–5</sup>. Drugs and hormones were found to affect the lipid microviscosity of biological membranes<sup>6,7</sup>, so it is plausible that their effect on electrical activity is mediated by changes in membrane fluidity. We report here that by changing the cholesterol content of *Aplysia* neurones, which presumably increases its membrane microviscosity<sup>8</sup>, long term, though reversible, alterations in electrical activity are induced.

In all experiments the visceral ganglion of *A. californica* was removed and the cell bodies were exposed by microdissection of the connective tissue. Microelectrodes filled with 3 M KCl were inserted into a neurone and its electrical activity was monitored using standard intracellular recording techniques<sup>9</sup>. Sonicated liposomes were used as exogenous pools for enrichment or depletion of the cell membrane cholesterol<sup>10,11</sup>. For preparation of cholesterol-rich liposomes 160 mg of egg lecithin (Sigma type VE) and 100 mg of cholesterol (Sigma CHS) in chloroform were mixed in a sonication vessel and evaporated to complete dryness under argon. Ten ml of seawater was added and the mixture subjected to 70 W ultrasonic irradiation (Branson Sonifier W350) for 60 min at 0 °C in an argon atmosphere. The liposomal dispersion was then centrifuged at 60,000g for 20 min and the bottom and upper layers were discarded. The middle layer, which presumably consisted primarily of single walled liposomes<sup>12</sup>, was used in the cholesterol enrichment experiments. For cholesterol depletion, liposomes of egg lecithin (160 mg in 10 ml seawater) were prepared analogously to the lecithin-cholesterol liposomes.

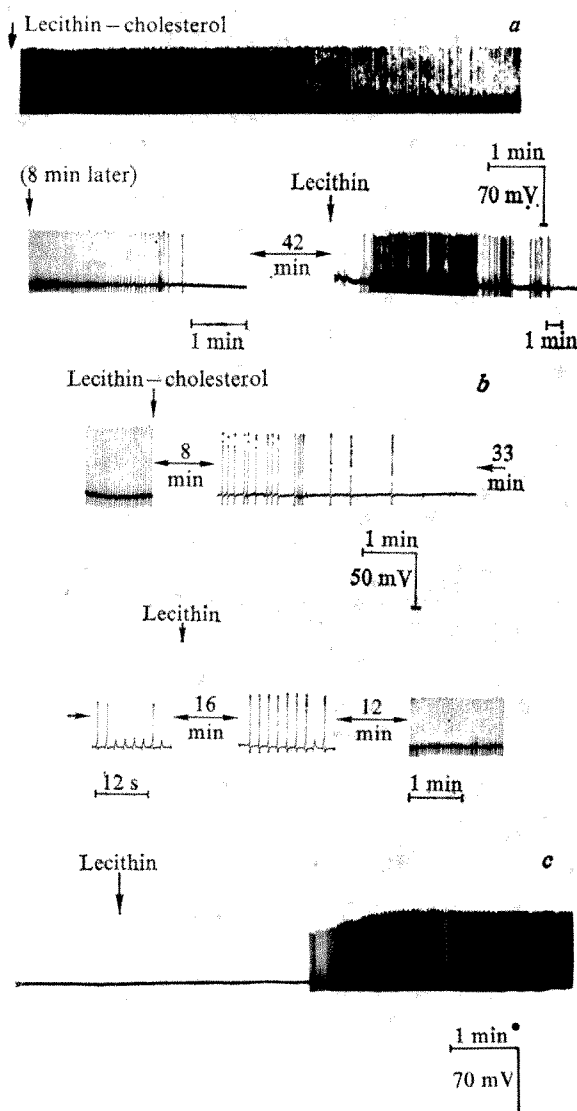
A freshly prepared liposome suspension was diluted 1:4 (v/v) in seawater and then applied to the ganglion by perfusion during continuous electrical recording for up to 3 h. In a series of 17 independent experiments perfusion of pacemaker and burster type neurones with lecithin-cholesterol liposomes was always found to gradually inhibit the spontaneous electrical activity of the neurones after a 4–10 min lag. After further 10–30 min all spontaneous activity ceased (Fig. 1a). Simultaneously, the threshold to elicit an action potential following intracellular stimulation progressively increased, in some cases reaching the stage where an action potential could not be produced by intracellular stimulation. Both the resting membrane potential and the steady-state slope conductance ( $\Delta I/\Delta E$ ) of the *Aplysia* neurones were apparently not affected by the lecithin-cholesterol perfusion. When active neurones were perfused with lecithin liposomes, no decrease in activity was observed.

For estimation of the rate of cholesterol uptake analogous liposomes made from egg lecithin and <sup>3</sup>H-cholesterol were prepared and applied separately to four isolated ganglia of similar size. The treated ganglia were thoroughly washed after 5, 15, 30, and 60 min of incubation, respectively, dissolved in a tissue solubiliser (NCS, Amersham) and then counted in a scintillation counter. Because of the large excess of the exogenous cholesterol in the incubation medium the rate of cholesterol depletion was considered as negligible compared to the rate of cholesterol uptake by the cells, which is therefore proportional to the accumulated radioactivity. The results showed a progressive uptake of cholesterol at a rate of approximately 300 pmol per h per ganglion. Assuming that the ganglion is composed of  $2 \times 10^3$  cells averaging about 200  $\mu$ m in diameter<sup>13</sup> which are partially exposed to the lecithin-cholesterol liposomes, and that the remaining glia cells do not interfere with the cholesterol uptake, then the observed rate of

cholesterol enrichment is of the same order of magnitude as in blood cells.<sup>10</sup>

The spontaneous electrical activity of the cholesterol-enriched neurones could be restored on subsequent perfusion with lecithin liposomes for less than 30 min (Fig. 1a,b). This treatment, which presumably depletes the membrane cholesterol in a similar fashion to that observed in other cells<sup>10,11</sup>, demonstrates that the effect of cholesterol is reversible and passive. In some cases, the restored spontaneous electrical activity lasted until the electrode was withdrawn (over 1 h). In other experiments, however, spontaneous activity ceased after 10–20 min without affecting the capacity of the neurone to produce an action potential after intracellular stimulation. Furthermore, perfusion with lecithin liposomes of *Aplysia* neurones which were initially silent, generated continuous action potentials similar to pacemaker activity (Fig. 1c) which in most cases lasted until the experiment was terminated (over 1 h).

**Fig. 1** Effect of liposome treatment on electrical activity of *Aplysia* neurones. *a*, Perfusion with lecithin-cholesterol liposomes ( $4 + 2.5$  mg ml<sup>-1</sup> in seawater) (commenced at arrow) caused spontaneous activity to cease. The ganglion was then washed in seawater and perfused with lecithin liposomes ( $4$  mg ml<sup>-1</sup> in seawater) which progressively restored electrical activity. The spontaneous activity was not maintained in this case. *b*, An identical experiment to (*a*) but with a separate ganglion. The response to a constant intracellular stimulation between the wash and after treatment with lecithin is shown. In this case, the restored spontaneous activity continued over 1 h when the electrode was withdrawn. *c*, Treatment with lecithin liposomes ( $4$  mg ml<sup>-1</sup> in seawater) of an initially silent neurone. The activity continued until the experiment was terminated (over 1 h). All experiments were carried out at 25 °C.



One of the main factors which determine the lipid microviscosity of cell membranes is the mole ratio of cholesterol to phospholipids (C/PL). In *in vitro* conditions this determinant can be modulated by treatment with liposomes by two main mechanisms. First, in the translocation mechanism, cholesterol molecules partition between the lipid pools of the liposomes and the membrane towards equilibration of their C/PL levels. This takes place in treatment of erythrocytes<sup>10</sup>, lymphocytes<sup>11</sup> and platelets<sup>14</sup> with liposomes of various C/PL. Second, the fusion mechanism, involves integration of the liposomes by fusion with the cell membrane<sup>15</sup>, presumably through specific receptors<sup>16</sup>. The fusion mechanism was demonstrated in a series of cells including lymphocytes<sup>17</sup> and fibroblasts<sup>18</sup>. In general, these two mechanisms do not affect the viability of cells and eventually lead to a similar change in C/PL of the cell membrane. Because of technical difficulties, we have been able to establish which mechanism operates in the perfusion of the *Aplysia* neurones with liposomes.

The common methods for measurements of membrane microviscosity<sup>8</sup> could not be applied to the isolated *Aplysia* ganglia. But, it is reasonable to assume that, as for most other cell membranes, enrichment with cholesterol markedly increases the microviscosity<sup>8</sup>, as well as the degree of exposure of membrane proteins<sup>19</sup>, and decreases the turnover of individual membrane enzymes and transport channels. The net effect of these modulations is expressed in alterations of cellular activity<sup>20</sup>. The findings presented here suggest that the activity of membrane sites, which are associated with spontaneous electrical activity and generation of action potential, is also dependent on membrane fluidity as determined by the cholesterol level. In principle, decrease in membrane fluidity can also be achieved by reducing the temperature. It has been previously shown<sup>2</sup> that by lowering temperature, the activity of *Aplysia* neurones is reduced, but to a lesser extent than with cholesterol enrichment. Possible alteration of the Na, K ATPase activity in the cholesterol enriched membrane<sup>21,22</sup> could be one of the factors which mediate the observed changes in electrical activity. As the resting membrane potential was not affected by the cholesterol enrichment or depletion, however, it seems that the electrogenic Na, K pump, which exerts a tonic contribution to the membrane potential in *Aplysia* neurones<sup>23</sup>, is not noticeably affected by membrane fluidity. Alteration in internal Ca activity could also contribute to the observed effects<sup>24,25</sup>. The lack of substantial changes in conductance and resting membrane potential upon enrichment with cholesterol suggests, however, that the major effect is on the channels and gates which are essential for action potential production<sup>26</sup>. Since the action potential height decreases during cholesterol treatment (Fig. 1b), and in some cases after prolonged perfusion only a small spread-out regenerative response could be elicited, it seems that inclusion of cholesterol decreases the active inward conductance. For a better understanding of the effect described here, voltage clamp and ion selectivity experiments are required.

The microviscosity of biological membranes may vary in various physiological and pathological conditions<sup>27</sup>. Neuroblastoma cells have been shown to undergo a marked modulation in membrane microviscosity during their cell cycle<sup>28</sup>. On differentiation of these cells the membrane fluidity is increased and this process can therefore be blocked by cholesterol enrichment<sup>29</sup>. By changing the lipid composition of neuroblastoma cells conspicuous morphological changes have been also observed<sup>30</sup>. Such changes may also occur in other nerve tissue, and may induce a significant alteration of neuronal activity *in vivo*.

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I. Johnston, D. & Ayala, G. *Science* **189**, 1009–1011 (1975).

2. Junge, D. & Stephens, C. *J. Physiol. Lond.* **235**, 155–181 (1973).
3. Klee, M., Faber, D. & Heiss, W. *Science* **179**, 1133–1136 (1973).
4. Paranas, I., Armstrong, D. & Strumwasser, F. *J. Neurophysiol.* **37**, 594–608 (1974).
5. Treisman, S. & Levitan, I. *Nature* **261**, 62–64 (1976).
6. Metcalfe, J., Seeman, P. & Burgen, A. *Molec. Pharmacol.* **4**, 87–95 (1968).
7. Jain, M., Wu, N. Y. & Wray, L. W. *Nature* **255**, 494–496 (1975).
8. Shinitzky, M. & Inbar, M. *Biochim. biophys. Acta* **433**, 133–149 (1976).
9. Stephens, C. *J. exp. Biol.* **58**, 411–421 (1973).
10. Cooper, R., Arner, E., Wiley, J. & Shattil, S. *J. clin. Invest.* **55**, 115–126 (1975).
11. Shinitzky, M. & Inbar, M. *J. molec. Biol.* **85**, 603–615 (1974).
12. Huang, C. & Thompson, T. E. *Methods Enzym.* **32**, 485–489 (1974).
13. Coggeshall, R. *J. Neurophysiol.* **30**, 1263–1287 (1967).
14. Shattil, S., Anaya-Galindo, R., Bennett, J., Colman, R. W. & Cooper, R. *J. clin. Invest.* **55**, 636–643 (1975).
15. Poste, G., Papadopoulos, D. & Vail, W. J. *Meth. cell. Biol.* **14**, 33–71 (1976).
16. Blumenthal, R., Weinstein, J. N., Sharrow, S. O. & Henkart, P. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
17. Huang, L., Ozato, K. & Pagano, R. E. *J. Memb. Biochem.* (in the press).
18. Papadopoulos, D., Poste, G., Vail, W. J. & Biedler, J. B. *Cancer Res.* **36**, 2988–2994 (1976).
19. Borochov, H. & Shinitzky, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4526–4530 (1976).
20. Shinitzky, M. *Bull. Schweiz. Akad. Med. Wiss.* **32**, 203–207 (1976).
21. Wiley, J. & Cooper, R. *Biochim. biophys. Acta* **413**, 425–431 (1975).
22. Giraud, F., Claret, M. & Garay, R. *Nature* **264**, 646–648 (1976).
23. Carpenter, D. & Alving, B. *J. gen. Physiol.* **52**, 1–21 (1968).
24. Meech, R. *Comp. Biochem. Physiol. A* **42**, 493–499 (1972).
25. Thomas, M. & Gorman, A. *Science* **196**, 531–533 (1977).
26. Hodgkin, A. & Huxley, A. *J. Physiol. Lond.* **117**, 500–544 (1952).
27. Cooper, R. *New Eng. J. Med.* **297**, 371–377 (1977).
28. deLaat, S., Van der Saag, P. & Shinitzky, M. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
29. deLaat, S., Van der Saag, P. & Shinitzky, M. *Biochim. biophys. Acta* (submitted).
30. Chen, J., Del Fa, A., Di Luzio, J. & Calissano, P. *Nature* **263**, 604–606 (1976).

## Asymmetry of phospholipid biosynthesis

THE asymmetrical distribution of phospholipids in the membrane of the red blood cell<sup>1–3</sup> and other membranes<sup>4,5</sup> is well documented. How this asymmetry arises in these membranes is an obvious question which remains unanswered. One explanation is that the enzymes that catalyse the last step in the synthesis of the phospholipids are unevenly distributed on both sides of the membrane where biosynthesis occurs. The phospholipids that are produced by these enzymes would be released on to each side of the lipid bilayer and thus account for lipid asymmetry. A necessary corollary would be that this asymmetry of the phospholipids would be preserved in the movement of lipids to other membranes in the cell. Initial reports on the lack of transbilayer movement of phospholipids in artificial membranes<sup>6,7</sup> and membranes of animal viruses<sup>8</sup> has made an uneven distribution of phospholipid biosynthetic enzymes an attractive hypothesis. Recent reports, however, have documented the transbilayer movement of phospholipids in the red blood cell membrane<sup>9–11</sup> and the membrane of *Bacillus megaterium*<sup>12</sup>. Although the mechanism for this transbilayer movement of phospholipids has yet to be elucidated, it is no longer necessary to explain lipid asymmetry by the uneven distribution of the phospholipid biosynthetic enzymes on both sides of the membrane. We summarise here our evidence that the enzymes that help to produce phosphatidylcholine and phosphatidylethanolamine are located exclusively on the cytoplasmic surface of the endoplasmic reticulum of rat liver and not on both sides of the membrane.

Subcellular fractionation of rat liver yields a microsomal fraction which consists largely of sealed fragments from the endoplasmic reticulum<sup>13</sup>. These vesicles retain the asymmetry of the endoplasmic reticulum as it exists in the cell; the exterior of the microsomal vesicles corresponds to the cytoplasmic surface of the endoplasmic reticulum, whereas the luminal surface is the interior of the microsomes<sup>13</sup>. Preparation of microsomes with the opposite orientation has not been reported.

A rapid method for evaluation of the permeability and orientation of microsomes by measurement of the latency of mannose-6-phosphatase has been reported<sup>14,15</sup>. The enzyme that hydrolyses mannose-6-phosphate is located inside the microsomes, which are apparently impermeable to low concentrations (1 mM) of this substrate. Treatment of microsomes with 0.4% taurocholate allows the substrate to penetrate the vesicles<sup>14</sup>. Thus, by assay of the mannose-6-phosphatase before and after treatment with taurocholate, one can assess the orientation and intactness of the membrane of the microsomal vesicles. The latency of this enzymatic activity is defined as the "activity in disrupted microsomes minus activity in untreated microsomes/activity in disrupted microsomes"<sup>15</sup>.

Microsomes from rat liver were prepared as described by Nordlie and Arion<sup>16</sup>, except that homogenisation was in 0.145 M NaCl and contaminating cytoplasmic enzymes were removed by passage of the microsomes once through a discontinuous sucrose gradient<sup>17</sup>. The microsomes were suspended in 0.145 M NaCl at approximately 7 mg protein ml<sup>-1</sup> and stored at -70 °C. The latency of mannose-6-phosphatase in all preparations was greater than 90%. The activities of CDP-choline:1,2-diacylglycerol cholinephosphotransferase and CDP-ethanolamine:1,2-diacylglycerol ethanolaminephosphotransferase were assayed essentially as described by Kanoh and Ohno<sup>18</sup>. The assays of cholinephosphate cytidyltransferase<sup>19</sup> and phosphatidylethanolamine-S-adenosylmethionine methyltransferase<sup>20</sup> followed published procedures.

In our initial experiments with the cholinephosphotransferase (which condenses CDP-choline and 1,2-diacylglycerol to form phosphatidylcholine), we found that treatment of the microsomes with 0.4% taurocholate resulted in a sixfold increase in the cholinephosphotransferase activity (from 3.3 to 19 nmol per min per mg protein). We did not know if this increase in enzyme activity were due to stimulation by the detergent or the expression of latent activity localised on the interior of the microsomes.

One approach for differentiation between these two possibilities was to digest the microsomes with a proteolytic enzyme and subsequently treat the microsomes with 0.4% taurocholate. Cholinephosphotransferase activity localised on the interior of the microsomes should survive this treatment. Rat liver microsomes (1.4 mg protein) were digested with bovine pancreatic trypsin (1 mg; Sigma), in 0.5 ml 50 mM Tris-HCl, pH 7.4, for 15 min at 30 °C. The reaction was stopped with the addition of 2 mg soybean trypsin inhibitor (Sigma). In control incubations the trypsin inhibitor was added before the trypsin, or isotonic saline was added instead of trypsin and trypsin inhibitor. Samples (0.18 ml) were removed and incubated with 20 µl 4% sodium taurocholate dissolved in 50 mM Tris-HCl, pH 7.4, or with buffer alone, for 30 min at 0 °C. The activity of cholinephosphotransferase was subsequently assayed. By this treatment the activity of the enzyme decreased from 19 to 0.5 nmol per min per mg protein. Since more than 97% of the cholinephosphotransferase was inactivated by trypsin, it seemed that none of the cholinephosphotransferase was localised on the interior of the microsomes.

It was important for us to demonstrate that during the trypsin digestion, the microsomes remained intact and impermeable to the trypsin. We therefore carried out an experiment in which the activity of the cholinephosphotransferase and the latency of the mannose-6-phosphatase were determined after incubation with trypsin for various times. The results in Fig. 1 clearly show that the latency of the mannose-6-phosphatase was not affected by the proteolytic digestion, whereas the activity of the cholinephosphotransferase was eliminated. Other studies have shown that the mannose-6-phosphatase is inactivated by trypsin in the presence of 0.4% taurocholate.

Similar studies were performed with CDP-ethanolamine:1,2-diacylglycerol ethanolaminephosphotransferase (which forms phosphatidylethanolamine), cholinephosphate cytidyltransferase (which forms CDP-choline) and phosphatidylethanolamine methyltransferase (which transforms phosphatidylethanolamine to phosphatidylcholine by the transfer of methyl groups from S-adenosylmethionine). The experiment in Fig. 1 shows that these enzymes are also inactivated by trypsin in conditions in which mannose-6-phosphatase activity is unaffected. It seems, therefore, that the enzymes that synthesise phosphatidylcholine and phosphatidylethanolamine are localised on the exterior surface of the microsomes.

There is an additional explanation of our results which should be considered. It is possible that the phospholipid biosynthetic enzymes extend across the bilayer of the microsomal membrane. Tryptic digestion of the part of the protein that is exposed to the exterior might inactivate the enzyme even if the active site were on the inside of the microsomal membrane. We are unaware of any example where there is this arrangement of a membrane-bound enzyme. Moreover, if the active site were on the interior of the microsomes, transport of the substrate into the microsomes must

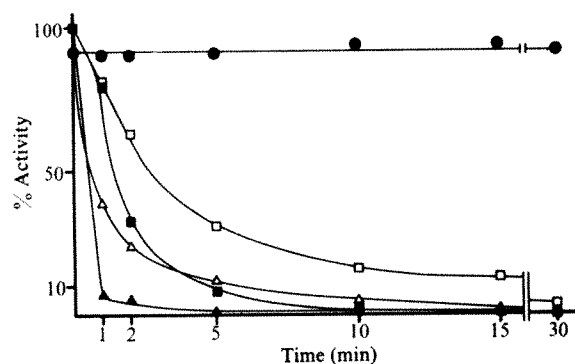


Fig. 1 Effect of trypsin on the activity of phospholipid biosynthetic enzymes associated with microsomes from rat liver. Microsomes (5 mg) were incubated with 3.5 mg trypsin, 10 mM Tris-HCl, pH 7.4, at 30 °C for the indicated time. Soybean trypsin inhibitor (5 mg) was added to terminate the reaction (final volume 1.5 ml). Aliquots from each incubation mixture were used to assay for cholinephosphotransferase<sup>18</sup> (■), ethanolaminephosphotransferase<sup>18</sup> (□), cholinephosphate cytidyltransferase<sup>19</sup> (▲) and phosphatidylethanolamine methyltransferase<sup>20</sup> (△) activities. Intactness of the microsomes was determined by measurement of the latency of mannose-6-phosphatase<sup>14,15</sup> (●).

occur. We have been unable to detect transport into the microsomes of any of the substrates for these phospholipid biosynthetic enzymes.

We conclude from our experiments that the enzymes that synthesise phosphatidylcholine and phosphatidylethanolamine are located on the cytoplasmic side of the endoplasmic reticulum. Thus, the origin of lipid asymmetry does not result from an uneven distribution of these enzymes on both sides of the endoplasmic reticulum. It remains a moot question as to how lipid asymmetry originates and is maintained in biological membranes.

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1. Bretscher, M. S. *J. molec. Biol.* **71**, 523-528 (1972); *Science* **181**, 622-629 (1973).
2. Zwaal, R. F. A., Roelofs, B. & Colley, C. M. *Biochim. biophys. Acta* **300**, 159-182 (1973).
3. Bretscher, M. S. & Raff, M. C. *Nature* **258**, 43-49 (1975).
4. Rothman, J. E. & Lenard, J. *Science* **195**, 743-753 (1977).
5. Bergelson, L. D. & Barsukov, L. I. *Science* **197**, 224-230 (1977).
6. Kornberg, R. D. & McConnell, H. M. *Biochemistry* **10**, 1111-1120 (1971).
7. Johnson, L. W., Hughes, M. E. & Zilversmit, D. B. *Biochim. biophys. Acta* **375**, 176-185 (1975).
8. Lenard, J. & Rothman, J. E. *Proc. natn. Acad. Sci. U.S.A.* **73**, 391-395 (1976).
9. Renooij, W., Van Golde, L. M. G., Zwaal, R. F. A. & Van Deenen, L. L. M. *Eur. J. Biochem.* **61**, 53-58 (1976).
10. Bloj, B. & Zilversmit, D. B. *Biochemistry* **15**, 1277-1283 (1976).
11. Renooij, W. & Van Golde, L. M. G. *FEBS Lett.* **71**, 321-324 (1976).
12. Rothman, J. E. & Kennedy, E. P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1821-1825 (1977).
13. Depierre, J. W. & Dallner, G. *Biochim. biophys. Acta* **415**, 411-472 (1975).
14. Aron, W. J., Ballas, L. M., Lange, A. J. & Wallin, B. K. *J. biol. Chem.* **251**, 4901-4907 (1976).
15. Aron, W. J., Lange, A. J. & Ballas, L. M. *J. biol. Chem.* **251**, 6784-6790 (1976).
16. Nordlie, R. C. & Aron, W. J. *Methods Enzym.* **9**, 619-625 (1966).
17. Brophy, P. J. & Gower, D. B. *Biochim. biophys. Res. Commun.* **360**, 252-259 (1974).
18. Kanoh, H. & Ohno, K. *Eur. J. Biochem.* **66**, 201-210 (1976).
19. Choy, P. C. & Vance, D. E. *Biochim. biophys. Res. Commun.* **72**, 714-719 (1976).
20. Rehlinger, D. & Greenberg, D. M. *Archs Biochem. Biophys.* **108**, 110-115 (1965).



## Light-sensitive membrane potentials in onion guard cells

INTRACELLULAR electrical recordings in onion guard cells show that they maintain a membrane potential difference (MPD), inside negative. The MPD is light-sensitive; cells subjected to short light and dark cycles depolarise in the dark and hyperpolarise in the light. The swiftness of the electrical changes makes them among the fastest known stomatal responses, suggesting a causal relationship between the reception of light, the changes in membrane potential and the ion fluxes known to be associated with stomatal movement. The magnitude and electrical sign of the responses are consistent with the activity of a light-sensitive proton pump which would provide the driving force for ion transport across the cell membrane. Guard cells, the specialised structures that control gas exchange in the leaves of higher plants, use potassium to modulate their intracellular osmotic potential and perform the mechanical work needed to open and close the stomatal pore<sup>1</sup>. When guard cells accumulate K<sup>+</sup> (with intracellular concentrations reaching up to 0.5 M (refs 2 and 3)), water content increases and swelling occurs, causing the pore to open. During the reverse process, K<sup>+</sup> is extruded (intracellular concentration diminishes to 0.1 M (refs 2 and 3)), leading to water loss and pore closure. Control of intracellular K<sup>+</sup> concentrations is a universal feature of living organisms. Nerve cells store energy used to generate electrical signals by maintaining concentration gradients of Na<sup>+</sup> and K<sup>+</sup> (ref. 4); bacterial cells selectively accumulate K<sup>+</sup> (ref. 5) and, in plants, cells expand by increasing their turgor through uptake of K<sup>+</sup> and its counter-ions<sup>6</sup>. While the cellular basis of K<sup>+</sup> uptake in stomatal guard cells is poorly understood, one might expect common mechanisms for K<sup>+</sup> transport even in widely divergent species. Two distinct mechanisms for K<sup>+</sup> transport have been defined. In one, common in animal cells, K<sup>+</sup> uptake is driven by ATP through a Na<sup>+</sup>/K<sup>+</sup> ATPase<sup>7</sup>; in the second system, demonstrated in bacteria, ion transport is conducted by a proton-motive force<sup>8</sup> generated by pH and electrical potential gradients across the membrane<sup>9</sup>. In showing that wall-less guard cell protoplasts swell when illuminated, we previously argued that a proton-motive force could drive K<sup>+</sup> uptake into the guard cells<sup>10</sup>. The hypothesis generates the basic prediction of a light-sensitive, inside negative, MPD across the guard cell membrane. We report here the experimental verification of that prediction, through intracellular recordings in guard cells of *Allium cepa*.

Both intact guard cells and cells with walls partially digested with a cellulolytic enzyme were used. Partial digestion of cells facilitated electrode penetration and gave us a basis for work on MPD and cell compartments, which are discussed elsewhere<sup>11</sup>. Slices from cotyledons of 7–14 d-old seedlings were the tissue source for both types of preparations<sup>12</sup>. The slices were adhered with a Valap mixture<sup>12</sup> to the bottom of a 5 ml well made in a 9.5-cm Petri dish filled with Sylgard (Dow Corning) and bathed with a 0.23 M mannitol solution containing 1 mM KCl and 1 mM CaCl<sub>2</sub>. The wall digestion was made with a 2% (w/v) solution of Cellulysin (Calbiochem) in the above solution for 2–5 h. At the end of the digestion, the preparation was washed twice with enzyme-free, bathing solution. Both types of preparations were dark adapted for 1–4 h before the experiments. Guard cells were impaled with glass capillary microelectrodes under direct visualisation at 400× magnification using a Zeiss compound microscope with a water-immersion objective. Microelectrodes were filled with 3 M KCl and bevelled after filling to final resistances of 40–90 MΩ. The signal from the microelectrode was put

through a high input impedance preamplifier and displayed on a cathode-ray oscilloscope and a slow speed chart recorder.

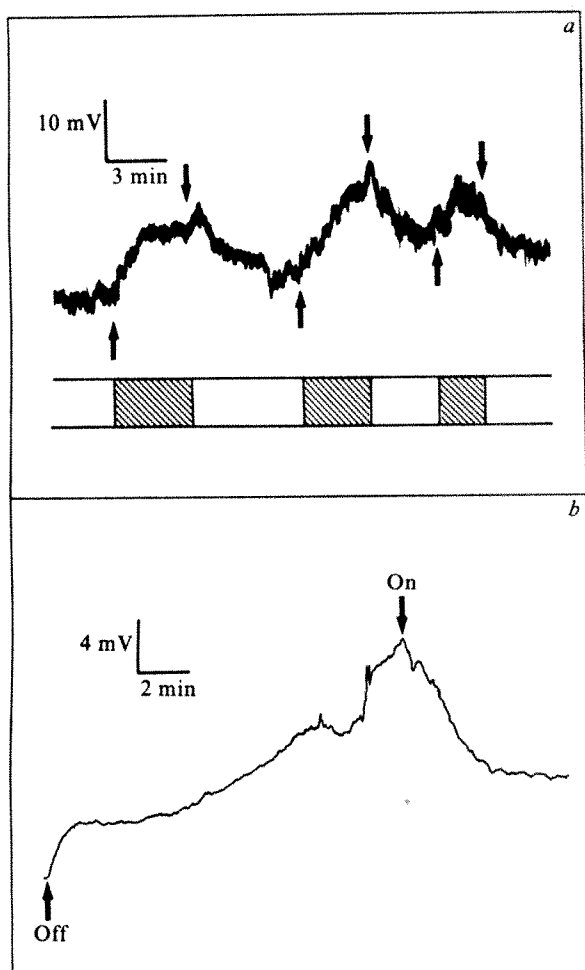
All guard cells showed MPD, inside negative. Partially digested cells averaged  $-39 \pm 7$  mV ( $n=65$ ) and intact guard cells  $-72 \pm 29$  mV ( $n=40$ ). Exposure of the cells to light cycles showed that the MPD is light-sensitive. Guard cells were impaled under dim green light, and those showing steady MPD ( $\pm 2$  mV) for at least 5 min, were subjected to alternating short dark and white light ( $200\text{--}500 \mu\text{E m}^{-2} \text{s}^{-1}$ ) cycles. The cells typically depolarised in the dark and hyperpolarised in the light (Fig. 1). Both intact and partially digested cells exhibited the electrical changes but the former had faster and larger responses. Depolarisations ranged between 1 and 60 mV; hyperpolarisations between 1 and 52 mV. The initial stages of the responses showed the highest rates, reaching as much as  $1.7 \text{ mV s}^{-1}$  for the depolarisations and  $0.9 \text{ mV s}^{-1}$  for the hyperpolarisations. Initial rates varied greatly, though, averaging about  $14 \text{ mV min}^{-1}$  for depolarisations and  $8 \text{ mV min}^{-1}$  for hyperpolarisations. After an initial fast phase, the responses become slower, saturating with approximately exponential kinetics (Fig. 1b). In addition, prolonged depolarisations showed a second, slow phase (Fig. 1b) which could not be observed in the hyperpolarisations. Actively responding cells showed alternating responses throughout 3–5 consecutive light/dark cycles with latencies ranging from 1–45 s (Fig. 1a). Typically, the depolarisations had shorter latencies.

It seems likely that these electrical responses of the guard cells are normal physiological events, associated with the K<sup>+</sup> fluxes seen during stomatal opening and closing. The short latencies and high initial rates of these light-induced membrane potential changes suggest that they could be an early and primary event in the mechanism controlling stomatal movement. The fact that the latencies of the dark responses, which we interpret as related to stomatal closing, are of the same order of magnitude as the latencies reported for closing in response to high CO<sub>2</sub> concentrations<sup>13</sup> also suggest a primary effect of light. The present results argue against the possibility that stomatal light responses are indirect and CO<sub>2</sub>-mediated<sup>14</sup>, since it is difficult to envisage that dark would cause a CO<sub>2</sub>-mediated response with the same latency as the CO<sub>2</sub> response alone. The data suggest, instead, that both dark and CO<sub>2</sub> can elicit a primary response leading to closing. Our observations showing that isolated guard cells from digested preparations also exhibit light-sensitive MPD indicate that light energy was absorbed by photopigments within the guard cell, without the mediation of other cells of the tissue.

An electrogenic mechanism driving K<sup>+</sup> transport in the guard cells has been suggested before<sup>1</sup> but the hypothesis has not become widely accepted because of negative reports on MPD across the guard-cell membrane<sup>2</sup> or its sensitivity to light<sup>15</sup>. The data presented here and related observations by Gunar *et al.*<sup>16</sup> give new support to the notion of an electrogenic pump being the driving mechanism of stomatal movement.

Light-induced electrical events associated with pump activity are well known in other systems. Vertebrate photoreceptors hyperpolarise when illuminated due to a change in membrane Na<sup>+</sup> conductance, probably mediated by the release of Ca<sup>2+</sup> from intracellular stores<sup>17</sup>. It is of interest that both onion guard-cell protoplasts<sup>10</sup> and outer-rod segments isolated from frog retinae<sup>18</sup> change their intracellular osmotic potentials in response to light. It seems plausible that the two cell types might share common mechanisms underlying phototransduction and ion fluxes.

Another useful system which provides insight on the basis of light-driven energy transduction coupled to ion transport has been described in *Halobacterium halobium*. In



**Fig. 1** Intracellular electrical recording in onion guard cells showing the changes in membrane potential differences in response to light and dark cycles. *a*, Three alternating dark/light cycles in an intact cell. Shaded portions in the abscissa show the dark periods, arrows point to the onset of each alternating stimulus. *b*, Dark and light response in a partially digested cell, seen at a higher gain. Both depolarisations and hyperpolarisations saturate with apparent exponential kinetics, with the depolarisations showing a second slower phase.

this microorganism a membrane-bound photopigment, bacteriorhodopsin, extrudes protons when illuminated, thus generating an electrochemical gradient across the cell membrane. This proton motive force can then be used to drive passive  $K^+$  influx<sup>19</sup> or to make ATP (ref. 20). In addition, ATP hydrolysis can be used to poise the proton gradient independently of illumination<sup>20</sup>, thus making the gradient a central device for the transduction of energy from either light or respiration<sup>21</sup>. Light-induced membrane hyperpolarisations ranging between 24–67 mV have been measured in *Halobacterium*<sup>22</sup>.

The demonstrated ability of a proton gradient to drive  $K^+$  uptake in the light and in the dark makes it a possible mechanism for energising ion transport during stomatal movement. Most of the known physiological properties of the guard cells seem consistent with the hypothesis and our findings of a light-sensitive MPD in onion guard cells adds new, though indirect, evidence.

The demonstration of a proton gradient across the guard-cell membrane as a primary energy-transducing mechanism would provide a link between the several possible energy sources used by the guard cells to drive ion fluxes. Blue light energy could be directly transduced as an electron flow when absorbed by a photoreceptor (a flavin?) which would be one of the links in the membrane-bound, electron transport chain which generates the

gradient. In addition, ATP hydrolysis would constitute a major energy source poising the gradient. ATP could be formed by oxidative phosphorylation in the guard cell mitochondria, which would be light independent and able to drive ion fluxes in the dark or by photophosphorylation in the guard cell chloroplasts which could utilise light energy with the spectral sensitivity of chlorophyll.

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1. Hsiao, T. C. in *Encyclopedia of Plant Physiology*, Part B (eds Lüttge, U. & Pitman, M. G.) 2, 195–221 (Springer, Berlin, 1976).
2. Sawhney, B. L. & Zelitch, I. *Pl. Physiol.* 44, 1350–1354 (1969).
3. Penny, M. G. & Bowling, D. J. F. *Planta* 119, 17–25 (1974).
4. Hodgkin, A. L. & Keynes, R. D. *J. Physiol. Lond.* 128, 28–60 (1955).
5. Harold, F. M. *Bact. Rev.* 36, 172–230 (1972).
6. Cram, W. J. in *Encyclopedia of Plant Physiology*, Part A (eds Lüttge, U. & Pitman, M. G.) 2, 284–316 (1976).
7. Hokin, L. E. & Dahl, J. L. in *Metabolic Pathways* (ed. Hokin, L. E.) 6, 270–315 (Academic, New York, 1972).
8. Mitchell, P. *Biol. Rev.* 41, 445–502 (1966).
9. Rhoads, D. B. & Epstein, W. *J. biol. Chem.* 252, 1394–1401 (1977).
10. Zeiger, E. & Hepler, P. K. *Science* 196, 887–889 (1977).
11. Moody, W. & Zeiger, E. *Planta* (in the press).
12. Zeiger, E. & Hepler, P. K. *Pl. Physiol.* 58, 492–498 (1976).
13. Raschke, K. *Pl. Physiol.* 49, 229–234 (1972).
14. Raschke, K. *A. Rev. Pl. Physiol.* 26, 309–340 (1975).
15. Pallaghy, C. K. *Planta* 80, 147–163 (1968).
16. Gunar, I. I., Zlotnikova, I. F. & Panichkin, L. A. *Sov. Pl. Physiol.* 22, 704–707 (1975).
17. Hagins, W. A. *A. Rev. Biophys. Bioengin.* 1, 131–158 (1972).
18. Brodie, A. E. & Bownds, D. *J. gen. Physiol.* 68, 1–11 (1976).
19. Garty, H. & Caplan, S. R. *Biochim. biophys. Acta* 459, 532–545 (1977).
20. Bogomolni, R. A. *Fedn Proc.* 36, 1833–1839 (1977).
21. Skulachev, V. *FEBS Lett.* 74, 1–9 (1977).
22. Michel, H. & Oesterhelt, D. *FEBS Lett.* 65, 175–178 (1976).

## Unprotonated chromophore-protein bond in visual pigments from $^{13}\text{C}$ -NMR spectra

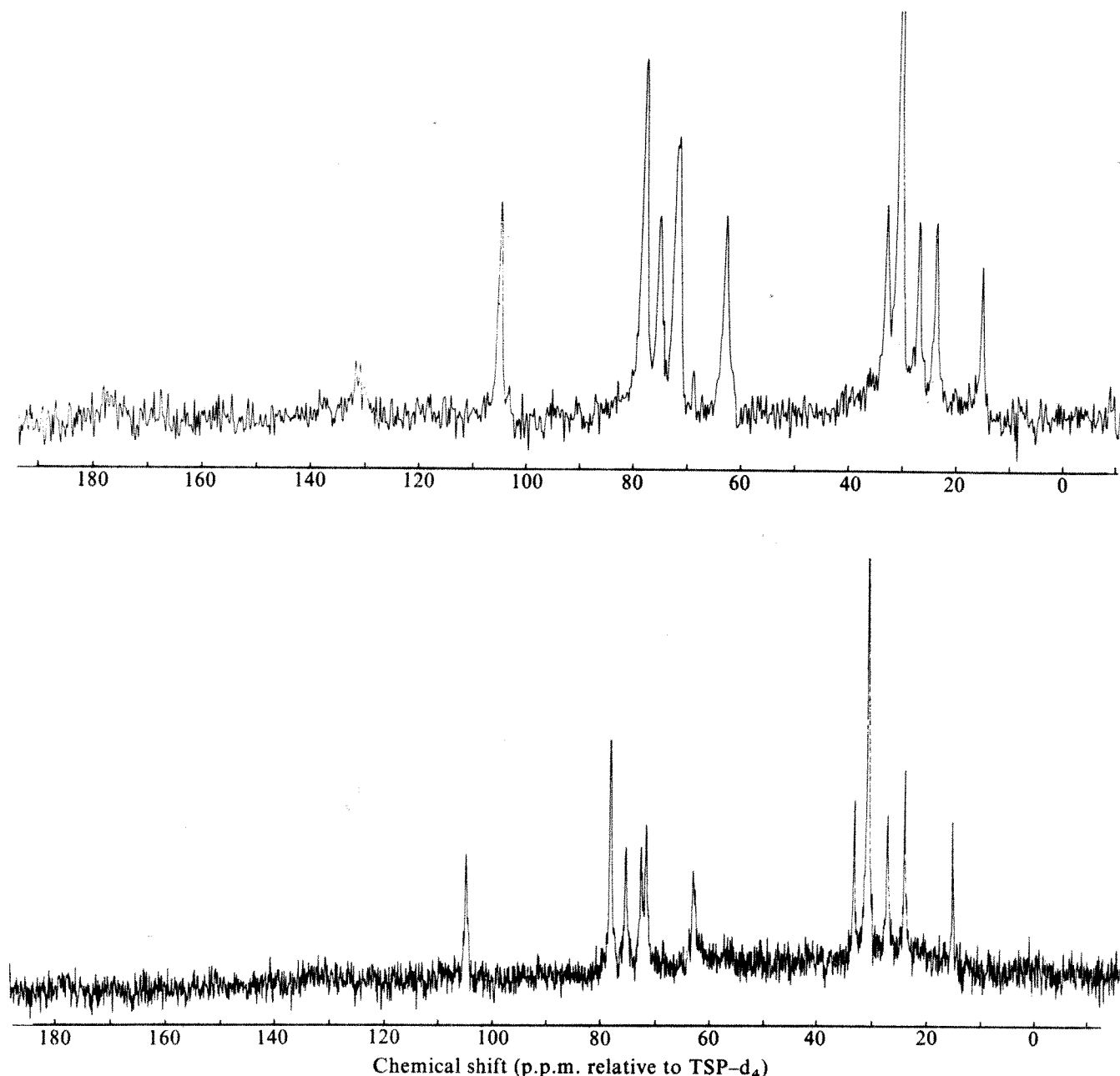
THE chromophore of visual pigments is generally assumed to be a protonated Schiff base of the 11-*cis* retinylidene (rhodopsins) or the 11-*cis* 3-dehydroretinylidene (porphyropsins) groups linked through a lysine amino group to a specific glycolipoprotein moiety (opsin)<sup>1</sup>. This protonated Schiff base hypothesis has been supported by plausible though not unequivocal chemical evidence<sup>2,3</sup>. Recent studies using the physical technique of resonance Raman spectroscopy have also seemed to support this view<sup>4–7</sup>. In an attempt to confirm this assignment and to explore further the structure and microenvironment of the chromophore, we have studied the nuclear magnetic resonance (NMR) spectra of aqueous suspensions of bovine rhodopsin which contain the chromophore enriched with  $^{13}\text{C}$  at specific positions in the retinylidene carbon chain. We report here our findings which clearly support an unprotonated retinylidene Schiff base as the visual chromophore in contrast to the earlier chemical and physical evidence which supports a protonated form.

Retinal isomers were synthesised enriched at the C14 position

from the C18 ketone<sup>8</sup> and triethyl phosphono[2-<sup>13</sup>C]acetate<sup>9</sup>. This latter compound was synthesised from triethylphosphite and ethyl [2-<sup>13</sup>C] bromoacetate. The resulting ester was converted to the [14-<sup>13</sup>C] retinal isomers by LiAlH<sub>4</sub> reduction and MnO<sub>2</sub> oxidation. High pressure liquid chromatography was used to isolate the 11-*cis* isomer of [14-<sup>13</sup>C] retinal<sup>10</sup>. <sup>13</sup>C-labelled rhodopsin was prepared by regenerating freshly photobleached bovine disks with labelled 11-*cis* retinal. After adding hydroxylamine to complex the excess retinal, the rhodopsin was solubilised by adding 50 mM aqueous phosphate-buffered octyl glucoside. Purification and delipidation was accomplished by affinity chromatography on Concanavalin A-Sepharose<sup>11,12</sup> using 30 mM octyl glucoside as detergent. This solution was then concentrated to 1–2 mM and dialysed three times to remove small organic molecules. Nuclear magnetic resonance

(NMR) spectra were collected in the Fourier transform mode on a 14 kG home built (DT) spectrometer as well as a Varian XL-100.

Figure 1a shows the <sup>13</sup>C NMR spectrum of a solution of octyl glucoside-solubilised bovine [14(retinylidene)-<sup>13</sup>C] rhodopsin containing no free retinal isomers. Figure 1b shows a spectrum of a solution of octyl glucoside and unenriched rhodopsin. The spectrum of the enriched pigment contains one additional resonance at approximately 130.8 p.p.m. downfield from TSP-d<sub>4</sub>. This peak is more pronounced in Fig. 2, where the rhodopsin concentration is twice that in Fig. 1a and the <sup>13</sup>C-<sup>1</sup>H dipolar interactions have been removed with high power decoupling. The peak at 175 p.p.m. in Fig. 2 is due to resonances arising from the amide carbonyls of the protein, observed because of the long pulse delay in this experiment.



**Fig. 1a.** <sup>13</sup>C NMR spectrum at 25.16 MHz of a solution of [14 (retinylidene)-<sup>13</sup>C] rhodopsin (0.5 mM) in 0.1 M KH<sub>2</sub>PO<sub>4</sub> buffer (pH 7.0) with 100mM octyl glucoside. Acquisition time, 0.2 s; pulse delay, 0.2 s; 30° pulse angle; 12 mm tube; and 58,170 transients. An external lock was used; **b.** <sup>13</sup>C NMR spectrum at 25.16 MHz of a solution of rhodopsin (0.5mM) in 100 mM octyl glucoside in 0.01 MKH<sub>2</sub>PO<sub>4</sub> buffer with D<sub>2</sub>O added for a lock (pH before D<sub>2</sub>O addition, 7.0). Acquisition time, 0.8 s; pulse angle, 30°; 21 mm tube; 20,000 transients.

Previous work from our laboratory with model visual pigment chromophores, that is, 11-*cis*-retinal, N-11-*cis*-retinylidene-propylimine (NRPI), and N-11-*cis*-retinylidenepropyliminium ion (NRPI-H<sup>+</sup>), have shown that the chemical shift of the C14 position of the protonated Schiff base of retinal is at a significantly higher field (about 120 p.p.m.) than in free retinal and its unprotonated Schiff base (about 130 p.p.m.) (ref. 13 and J.W.S., G.D.M. and E.W.A., unpublished). Quite clearly, there is a large difference in chemical shift of the C14 position between that observed in <sup>13</sup>C-enriched rhodopsin and the model protonated Schiff base of retinal. It is difficult to account for this large difference in terms of microenvironmental interactions. All interactions, steric, coulombic, ring currents, and so on, which are associated with protein folding would generally not lead to chemical shift differences larger than 2 p.p.m.<sup>14-17</sup>.

We have found that salts of model Schiff bases of all-*trans* retinal show the chemical shift of the C14 carbon in a pigment with a visible absorption maximum at 498 nm should be even further upfield than 120 p.p.m. (Fig. 3). In addition, there is a very small variation in the observed chemical shift for model pigments absorbing from 428 to 480 nm in cases where the counterion is associated with the nitrogen atom.

We have calculated the variation of the C14 chemical shift as a function of various counterion placements along the polyene chain from Pariser-Parr-Pople electron densities using the Karplus-Pople formula (ref. 18 and J.W.S. *et al.*, unpublished). These show no anomalous downfield shifts to 130 p.p.m. in a model pigment which has a significant bathochromic shift (about 140 nm) relative to the unprotonated Schiff base. Presumably this is due to the proximity of C14 to the protonation site; a significant negative charge is forced on C14 with protonation, and its magnitude varies little with counterion placement. Therefore, the chemical shift of C14 in a protonated Schiff base chromophore absorbing near 500 nm must be approximately 120 p.p.m. downfield from TMS. Attempting to position a charge such that a chemical shift of 130 p.p.m. would be observed should lead to a long wavelength absorption maximum near 350 nm.

The use of two anionic charges, one placed near the protonated nitrogen atom such as proposed by Honig *et al.*<sup>19</sup> would also be expected to give shifts near 120 p.p.m. downfield from TMS for a pigment with a long wavelength absorption maximum near 500 nm since the C = N region in this case behaves similarly to the solution case. The above considerations clearly argue against the N-protonated retinylidene Schiff base model for the chromophore of rhodopsins.

It is most significant that the C14 shift for rhodopsin is consistent with the chromophore being an unprotonated Schiff

Fig. 2 <sup>13</sup>C NMR spectrum (14 KG field) of a solution of [14 (retinylidene-<sup>13</sup>C) rhodopsin (1.0 mM) in 0.1 M KH<sub>2</sub>PO<sub>4</sub> buffer (pH 7.0) with 100 mM octyl glucoside. Acquisition time, 0.2 s; pulse delay, 2.0 s; pulse angle, 90°; 8 mm tube; and 8092 transients. An external lock was used.

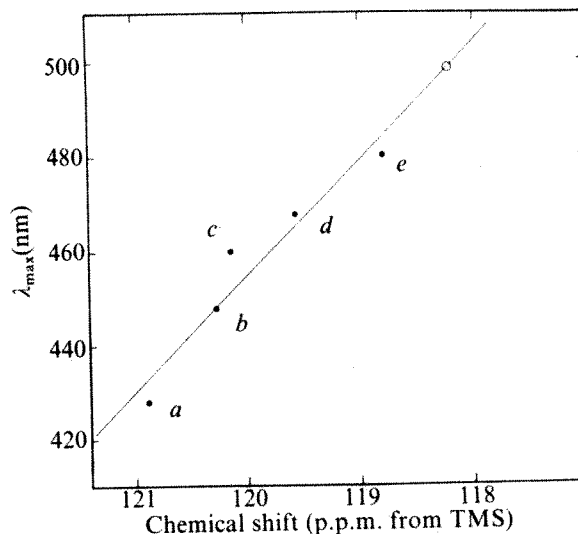
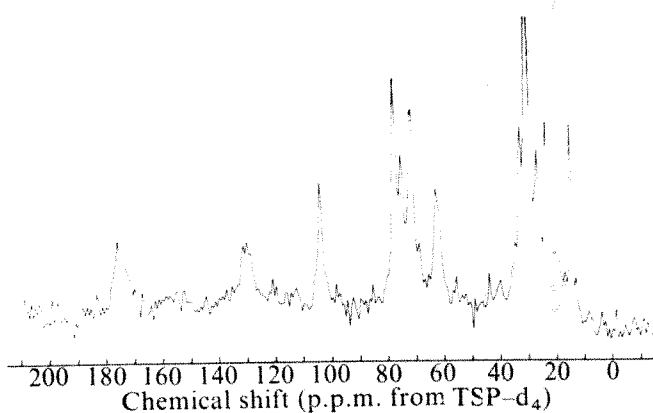


Fig. 3 Variation in shift of C14,  $\delta C14$ , as a function of the long wavelength absorption maximum of all-*trans*-retinylidenepropyliminium ions, a, NRPI-HCl in acetone; b, NRPI-HCl in EtOH; c, NRPI-HCl in CDCl<sub>3</sub>; d, NRPI-HBr in CDCl<sub>3</sub>; e, NRPI plus a 100-fold excess of TEA in CH<sub>2</sub>Cl<sub>2</sub>. ○, Predicted value for a pigment with an all-*trans* chromophore with  $\lambda_{max}$  = 498 nm is 118.26 p.p.m.

base of retinal ( $\delta C14$  = 129.96) or retinal itself ( $\delta C14$  = 129.99 p.p.m.). Chemical evidence indicates that the retinylidene chain is bound to an  $\epsilon$ -amino group of lysine on opsin rather than existing *in situ* as free retinal<sup>20, 21</sup>. This would lend support to the original notion of Dartnall of an unprotonated Schiff base linkage<sup>22</sup>.

The above NMR results run counter to the conclusions drawn from resonance Raman spectra of rhodopsin. The band assigned to  $\nu(C=N)$  of protonated Schiff base linkage by various investigators varies from 1645 to 1660 cm<sup>-1</sup> for rhodopsin having an absorption maximum at 498 nm<sup>13</sup>. Other peaks in the spectra—for example,  $\nu(C=C)$ —vary to a much lesser degree ( $\sim 4$  cm<sup>-1</sup>). Furthermore, bathorhodopsin and isorhodopsin both have a 1665 cm<sup>-1</sup> peak, whereas their visible absorption maxima are 543 and 483 nm, respectively; one would expect to find some variation of  $\nu(C=N)$  with absorption maxima of the chromophore. This line of reasoning, together with the NMR results presented here, suggests that the peak in the 1650 cm<sup>-1</sup> region may be incorrectly assigned.

In conclusion, we point out that the NMR spectra of <sup>13</sup>C-enriched rhodopsin leaves little question of the assignment of the probe signal while in resonance Raman spectra this is not the case. It seems evident from the results reported here that the Dartnall model of an unprotonated Schiff base chromophore in native visual pigments deserves further attention.

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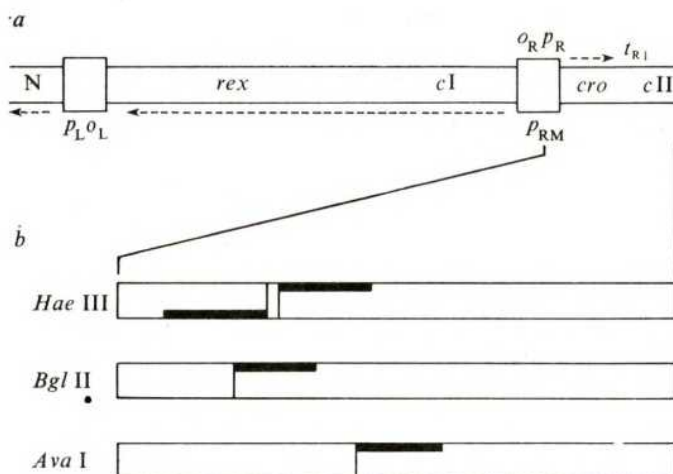
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1. Abrahamson, E. W. & Fager, R. S. *Curr. Top Bioenerget.* **5**, 125 (1973).
2. Morton, R. & Pitt, G. *Fortschr. Chem. Org. Naturst.* **14**, 244-316 (1957).
3. Hubbard, R. *Nature* **221**, 432 (1969).
4. Lewis, A., Fager, R. S. & Abrahamson, E. W. *J. Raman Spectrosc.* **1**, 465 (1973).
5. Oseroff, A. R. & Callender, R. H. *Biochemistry* **13**, 4243 (1974).
6. Mathies, R., Oseroff, A. R. & Stryer, L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1 (1976).
7. Callender, R. H., Doukas, A., Crouch, R. & Nakanishi, K. *Biochemistry* **15**, 1621 (1976).
8. Isler, O. ed. *Carotenoids*, (Basel, 1971).
9. Wadsworth, W. S., Jr & Emmons, W. D. *J. Am. chem. Soc.* **83**, 1723 (1961).
10. Rotmans, J. P. & Kropf, A. *Vision Res.* **15**, 1301 (1975).
11. Steineman, A. & Stryer, L. *Biochemistry* **12**, 1499 (1973).
12. Stubbs, G. W., Smith, H. G., Jr & Litman, B. J. *Biochem. biophys. Acta* **425**, 46 (1976).
13. Shriver, J. W., Abrahamson, E. W. & Mateescu, G. D. *J. Am. chem. Soc.* **98**, 2407 (1976).
14. Browne, D. T., Kenyon, G. L., Packer, E. L., Sternlicht, H. & Wilson, D. M. *J. Am. chem. Soc.* **95**, 1316 (1973).
15. Allerhand, A., Childers, R. F. & Oldfield, E. *Biochemistry* **12**, 1335 (1973).
16. Chaiken, I. M., Cohen, J. S. & Sokoloski, E. A. *J. Am. chem. Soc.* **96**, 4703 (1974).
17. Brewster, I. R., Hruby, V. J., Spatula, A. F. & Bavey, F. A. *Biochemistry* **12**, 1643 (1973).
18. Karplus, M. & Pople, J. A. *J. Chem. Phys.* **38**, 2803 (1963).
19. Honig, B., Greenberg, A. D., Dinur, U. & Ebrey, T. G. *Biochemistry* **15**, 4593 (1976).
20. Bownds, D. *Nature* **216**, 1178 (1967).
21. Abrahamson, E. W. *Biochem. biophys. Res. Commun.* **47**, 1244 (1972).
22. Dartnall, H. J. A. *The Visual Pigments* (Methuen, London, and Wiley, New York, 1957).

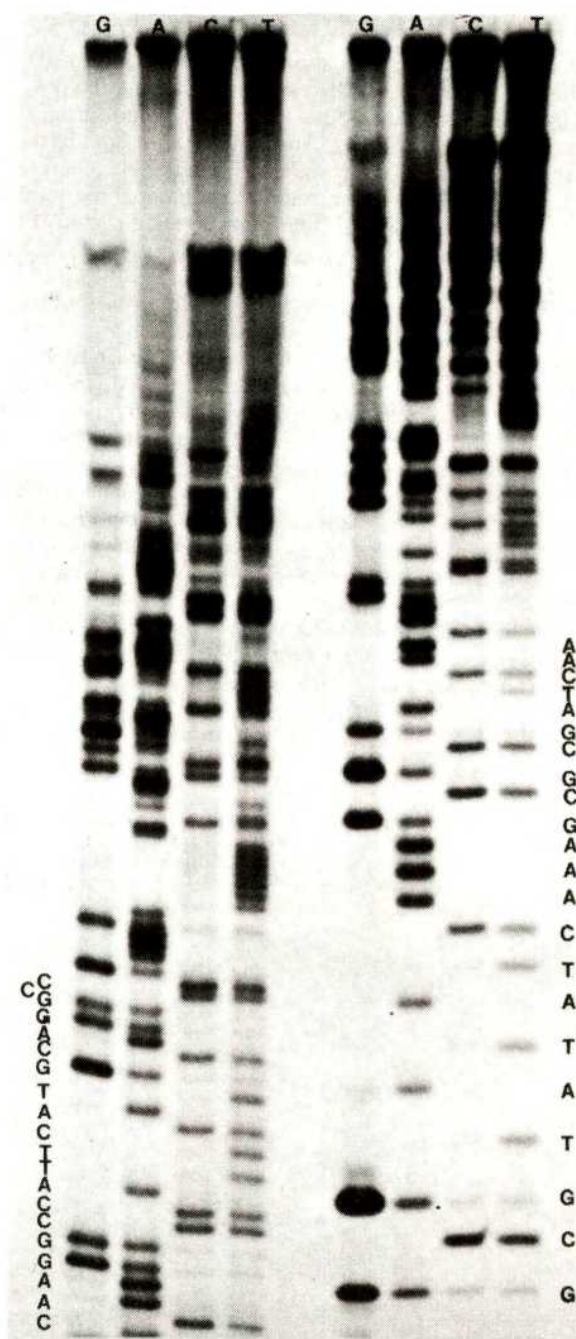
KNOWLEDGE of the primary sequences of repressor proteins and the DNA operators with which they interact is essential for detailed study of the molecular aspects of the control of gene expression. The sequences of two repressors are known: the cI protein of lambda (ref. 1 and R. Sauer and R. Anderegg, personal communication) and the *i* gene product of the lactose operon of *Escherichia coli* (refs 2,3 and P. J. Farabaugh, personal communication). Here we report the DNA sequence for the structural gene of a third repressor, the Cro protein of lambda. This protein is of special interest both because of its small size (66 amino acids, as compared with the 236 amino acids of the cI protein and the 360 amino acids of the *lac i* gene), and because genetic evidence suggests that it interacts with the same operator regions as does the cI protein, although the

**Fig. 1** *a*, Location of the *cro* gene within the immunity region of the lambda chromosome. *b*, An enlargement of the 550 base pair *Hin* II fragment which contains the *cro* gene showing the sites of restriction for three other endonucleases. The thickened lines indicate DNA strands which were end labelled and sequenced. The start point of the *cro* gene is 51 base pairs to the right of the left end of the *Hin* II fragment.



The location of the *cro* gene on the lambda chromosome is shown in Fig. 1a. DNA sequencing was carried out by direct chemical sequencing\* following the scheme of <sup>32</sup>P

**Fig. 2** Autoradiogram of a polyacrylamide gel displaying 5' end-labelled oligonucleotide products resulting from partial, base-specific cleavage of the DNA fragment extending to the right of the *Bgl* II site shown in Fig. 1b. To sequence this region of the gene, DNA from a plasmid (A. Johnson, unpublished) containing the portion of the lambda chromosome extending from the rightmost *Hin* III site in the *cl* gene to the *Eco* RI site in the *o* gene was cut with *Bgl* II, dephosphorylated with bacterial alkaline phosphatase, and 5' end-labelled using [ $\gamma$ - $^{32}$ P]ATP and T4 polynucleotide kinase. The resulting labelled fragments were digested with *Hin* II, and separated electrophoretically on a 3.5% polyacrylamide gel. The 440-base pair *Bgl* II-*Hin* II fragment containing the carboxy-portion of the *cro* gene was isolated from the gel and partially sequenced by the chemical procedures of Maxam and Gilbert<sup>8</sup>.



end labelling shown in Fig. 1b. Figure 2 shows an autoradiogram of a typical sequencing gel. The completed DNA sequence is shown in Fig. 3. Wherever possible the DNA sequence was checked by comparison with a catalogue of sequenced oligoribonucleotides generated by both ribonuclease T<sub>1</sub> and pancreatic RNase digestion of the RNA transcript of the region from *P<sub>R</sub>* to *t<sub>RI</sub>* synthesised *in vitro*. All oligoribonucleotides larger than tetramers have been uniquely identified. In this manner independent confirmation was obtained for over 70% of the sequence. The initial 27 bases of the sequence are in agreement with the sequence predicted for the 5' end of *cro* by Steege and Steitz<sup>9</sup> from RNA sequencing of the message initiated at *P<sub>R</sub>*.

Takeda *et al.*<sup>10</sup> have isolated, identified, and determined the protein sequence of the lambda Cro protein. Their amino acid sequence is in exact agreement with that predicted by our DNA sequence. The occurrence of an ochre termination codon immediately beyond the COOH terminal alanine residue indicates that the cellular form of Cro is not processed. Comparison of the amino acid sequence determined for Cro with that of the lambda repressor indicates that no obvious similarities exist in the amino acid sequences of the two proteins.

Several aspects of the *cro* sequence are worth noting. (1) The 5' ATG of our sequence is three bases to the right of the putative ribosome binding site found by Steege and Steitz in the leader of the *cro* message<sup>9</sup> and is 18 bases from the start point of transcription. (2) There is a region of perfect twofold symmetry in the DNA sequence involving bases 130–138 and 145–153. This hyphenated symmetry would give rise to a potential stem and loop structure in the RNA transcript of this region. The significance of this feature is not known; however, it does share the sequence ATAAA . . . . . TTTAT with the right operator region *O<sub>R</sub>*. (3) The region from base 106 to base 125 consists of a G-C rich region (106–113) followed at a short distance by the sequence TTTT TTA (Fig. 2). This primary structure is reminiscent of known sites of *rho* independent transcription termination<sup>11</sup>. But, *in vitro* transcription studies indicate that in the absence of additional factors RNA polymerase does not terminate transcription at this site (M.R., unpublished). (4) Finally, the distribution of bases utilised in the third or 'wobble' position of the codons of *cro* is approximately random (there is a slight bias against G's and toward A's and C's). There are, however, pronounced A-T or G-C rich regions within the *cro* sequence (for example, the region between positions 114 and 134 is

91% A-T; the region between 168 and 179 is 75% G-C; and the region between 180 and 201 is 77% A-T).

It should be noted that the region of the lambda genome which encodes the Cro protein apparently serves as template for leftward transcription (from the opposite strand) into the *cI* gene during establishment of repressor synthesis relatively early during phage development<sup>12</sup>. Thus, the structural features noted above may reflect regulatory information involved in this transcription while concomitantly specifying the appropriate amino acid sequence for a functional Cro protein.

We thank M. Ptashne for helpful discussions, A. Johnson for making available plasmids containing *cro* gene and Alik Honigman for purified *AvaI* endonuclease. This work was supported in part by grants from the US NIH and NSF to M.P. T.M.R. is supported by an NIH postdoctoral fellowship.

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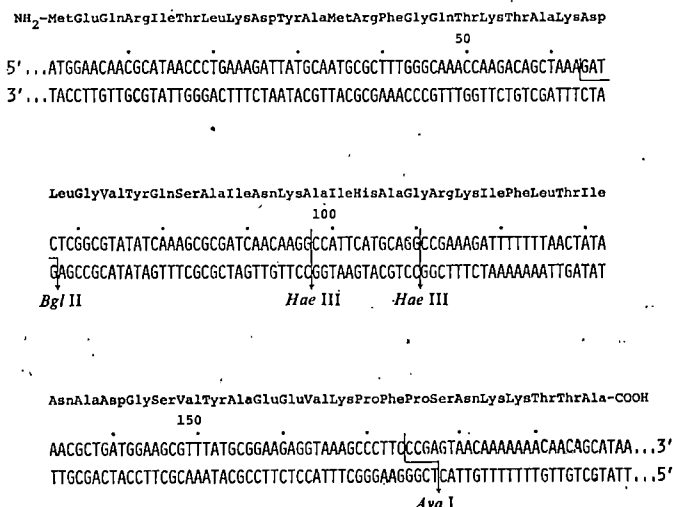
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1. Ptashne, M. *et al. Science* 194, 156–161 (1976).
2. Gilbert, W., Maizels, N. & Maxam, A. *Cold Spring Harb. Symp. quant. Biol.* 38, 845–855 (1974).
3. Beyreuther, K., Adler, K., Geisler, N. & Klemm, A. *Proc. natn. Acad. Sci. U.S.A.* 70, 3576–3580 (1973).
4. Pero, J. *Virology* 40, 65–71 (1970).
5. Sly, W. S., Rabideau, K. & Kolber, A. in *The Bacteriophage Lambda* (ed. Hershey, A. D.) 575–588 (Cold Spring Harbor Laboratory, 1971).
6. Berg, D. *V. ology* 62, 224–233 (1974).
7. Reichardt, L. *J. molec. Biol.* 93, 267–288; 289–301 (1975).
8. Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* 74, 560–564 (1977).
9. Steege, D. & Steitz, J. *J. molec. Biol.* (in the press).
10. Takeda, Y., Hsiang, M., Cole, D. & Echols, H. *Nature* 270, 275–277 (1977).
11. Gilbert, W. in *RNA Polymerase* (eds Losick, R. & Chamberlin, M.) 193–203 (Cold Spring Harbor Laboratory, 1976).
12. Spiegelman, W. G., Reichardt, L., Yaniv, M., Heineman, S. F., Kaiser, A.D. & Eisen, H. *Proc. natn. Acad. Sci. U.S.A.* 69, 3156–3160 (1972).

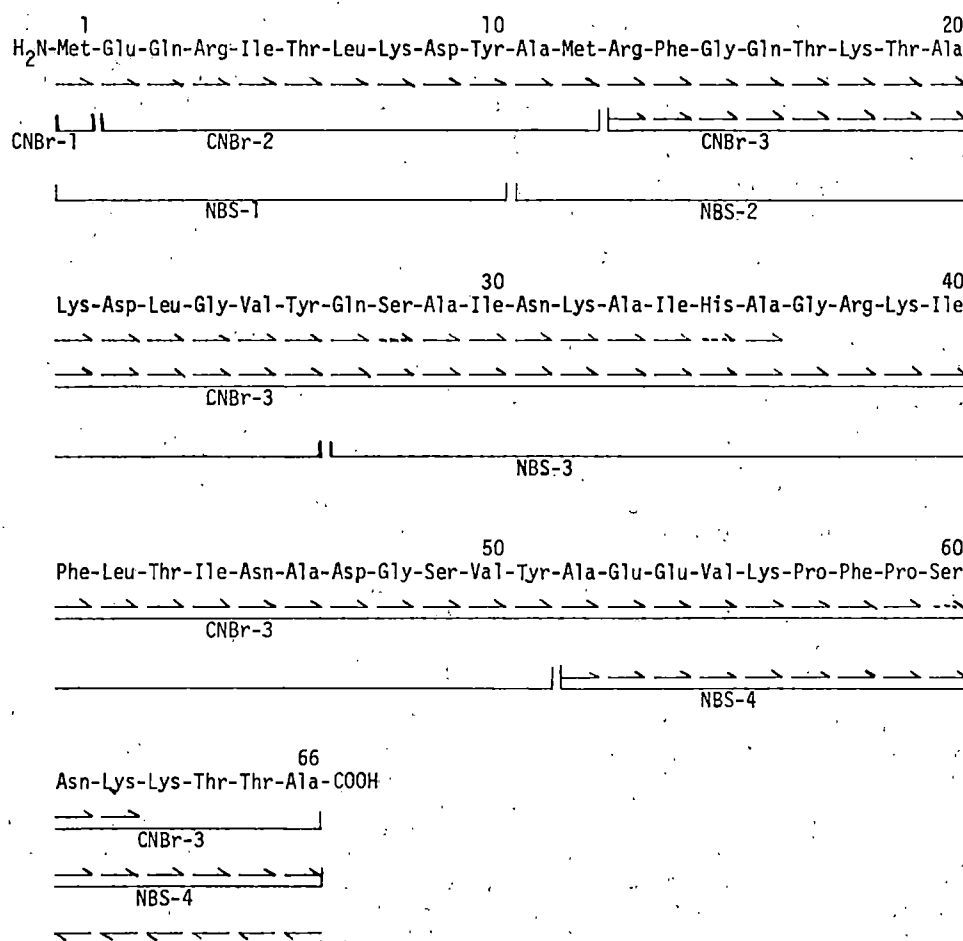
Fig. 3 DNA and protein sequences of the *cro* gene.



## Amino acid sequence of Cro regulatory protein of bacteriophage lambda

THE Cro protein specified by bacteriophage lambda is a repressor of the genes expressed early in phage development; this temporal regulation is required for a normal late stage of lytic growth (see refs 1–5). Cro protein has been purified and shown to bind to the operator regions, *O<sub>L</sub>* and *O<sub>R</sub>*<sup>6,7</sup>, used by the *cI* repressor protein to maintain lysogeny<sup>8</sup>; purified Cro also represses RNA synthesis initiated at the early promoter sites on  $\lambda$  DNA<sup>7</sup>. Among the features that make Cro interesting for further biochemical study are its timed physiological action<sup>1–5</sup> and apparent role in regulation of DNA replication<sup>9</sup>. Cro is also convenient for structural analysis because of its extremely small size<sup>6,7</sup>. Here we report the amino acid sequence of Cro protein.

The complete amino acid sequence of Cro protein has been determined by Edman degradation<sup>10</sup> of the intact protein, and two series of peptide fragments, one derived from cyanogen bromide



**Fig. 1** The amino acid sequence of Cro protein of bacteriophage lambda. Peptides are designated as CNBr when derived from cleavage by cyanogen bromide or as NBS when derived from *N*-bromosuccinimide cleavage; they are numbered sequentially in the order which they occur in the protein. Residue positions determined by automatic Edman degradation<sup>10</sup> (→) or by hydrolysis with carboxy-peptidases<sup>20</sup> (←) are indicated with arrows. Amino acid sequence analysis was performed by using a Beckman automatic sequencer 890C and the Beckman DMAA peptide program. Identification of PTH-amino acids was by (1) gas-liquid chromatography<sup>17</sup> on a Varian gas chromatograph 1840 A; (2) high-pressure liquid chromatography<sup>18</sup> on a Waters Associates liquid chromatograph with  $\mu$ Bondapak C<sub>18</sub> column; (3) amino acid analysis after HI hydrolysis<sup>19</sup>.

cleavage and the other from *N*-bromosuccinimide (NBS) cleavage. Digestion of the whole protein by carboxypeptidases A and B<sup>20</sup> further confirmed the sequence on the carboxyl end. The results are shown in Fig. 1 and Table 1.

The proposed structure contains 66 amino acid residues with a calculated molecular weight of 7,351 and has methionine and alanine as its amino and carboxyl termini respectively. The primary structure is in complete agreement with the amino acid

**Table 1** Amino acid compositions and other properties of peptides of Cro protein

	CNBr-1	CNBr-2	CNBr-3	NBS-1	NBS-2	NBS-3	NBS-4
Lys	0.22	1.12(1)	7.00(7)	1.33(1)	2.12(2)	2.19(2)	3.11(3)
His			0.86(1)			0(1)**	
Arg		1.14(1)	2.09(2)	1.00(1)	1.10(1)	1.00(1)	0.10
Asp	0.38	1.23(1)	5.20(5)	1.39(1)	1.47(1)	3.08(3)	1.32(1)
Thr		0.83(1)	4.83(5)	0.91(1)	1.87(2)	1.30(1)	2.07(2)
Ser	0.61	0.12	2.84(3)		0.40	1.73(2)	1.06(1)
Glu		2.15(2)	4.69(4)	1.92(2)	1.47(1)	1.35(1)	2.26(2)
Pro			2.04(2)				2.00(2)
Gly	0.46	0.24	4.02(4)	0.65	2.16(2)	2.26(2)	0.41
Ala		1.31(1)	7.22(7)	0.35	2.40(2)	3.89(4)	2.34(2)
1/2 Cys							
Val			3.01(3)		0.93(1)	1.11(1)	0.96(1)
Met	1.0†(1)	0.87‡(1)		0.30(1)	0.71(1)		
Ile		1.01(1)	3.84(4)	0.75(1)	0.56	3.80(4)	0.29
Leu		1.05(1)	2.19(2)	1.09(1)	1.16(1)	1.24(1)	0.10
Tyr		0.76(1)	1.84(2)	0(1)¶	0(1)¶	0(1)¶	
Phe			2.94(3)		0.71(1)	1.06(1)	0.80(1)
Total residue	1	11	54	10	16	25	15
Yield (%)	36.1	35.0¶	87.5	72.0	62.0	38.0	18.0
Purification steps*	A	A	A	A	AC	ABC	ABCD
Treatment of peptides†			E				F, G

\*Purification steps, given in order of application. A, Gel filtration by Sephadex G-50 superfine; B, paper electrophoresis pH 6.4; C, paper electrophoresis pH 3.5; D, paper chromatography

†Treatment of peptide before sequencing. E, 4-sulphophenylisothiocyanate<sup>21</sup>; F, 2-amino-1,5 naphthalene disulphonic acid and *N*-ethyl, *N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride<sup>22</sup>; G, acetylated cytochrome c (3 mg) was added in the sequencer cup to reduce the loss of peptide during automatic sequencing.

‡As homoserine

¶Low yield due to loss in handling

|| Detected by absorption of spirolactone

\*\*Brominated

sequence predicted from the DNA sequence of the *cro* gene (ref. 11; and G. Hobom, personal communication). The composition of Cro protein based on the proposed sequence is Asp<sub>3</sub>Asn<sub>3</sub>Thr<sub>6</sub>Ser<sub>3</sub>Glu<sub>3</sub>Gln<sub>3</sub>Pro<sub>2</sub>Gly<sub>4</sub>Ala<sub>8</sub>Val<sub>3</sub>Met<sub>2</sub>Ile<sub>5</sub>Leu<sub>3</sub>Tyr<sub>3</sub>Phe<sub>3</sub>Lys<sub>8</sub>His<sub>3</sub>Arg<sub>3</sub>, which is 12 amino acid residues less than the composition previously reported<sup>7</sup>. This difference is largely due to renormalisation. Careful reanalysis of Cro protein of the highest purity and renormalisation (based on alanine) gives a composition that agrees with the one calculated from the sequence within experimental error.

The rules of Chou and Fasman<sup>12,13</sup> have been applied to the amino acid sequence of Cro protein; these predict several alternating regions of  $\alpha$ -helix and  $\beta$ -sheet, consistent with an overall globular conformation.

Although Cro and cI proteins recognise the same operator regions of  $\lambda$  DNA, there is no extensive sequence homology between the two proteins<sup>14</sup>. This indicates that the two repressors were derived by parallel evolution rather than by partial duplication of one of the two genes. The very different chemical nature of the two repressors probably reflects the different physiological needs of regulation during lytic development or lysogeny<sup>1,2,7</sup>. Substantial biochemical differences have already been shown in the affinity of the two proteins for operator DNA<sup>7,8,15</sup> and in the effect of operator region mutations on their binding properties<sup>6</sup>. There is also no detectable sequence homology between Cro protein and the eukaryotic chromosomal protein, histone H2B<sup>16</sup>, despite the close resemblance of their amino acid compositions.

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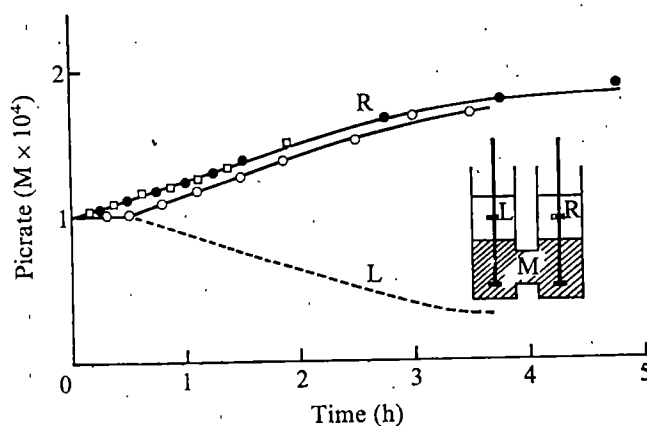
Received 11 August; accepted 2 September 1977.

1. Echols, H. A. *Rev. Genet.* **6**, 157-190 (1972).
2. Herskowitz, I. A. *Rev. Genet.* **7**, 289-324 (1973).
3. Reichardt, L. J. *molec. Biol.* **93**, 267-288; 289-309 (1975).
4. Takeda, Y., Ogata, K. & Matsubara, K. *Virology* **65**, 385-391 (1975).
5. Takeda, Y., Matsubara, K. & Ogata, K. *Virology* **65**, 374-384 (1975).
6. Folkmanis, A., Takeda, Y., Simuth, J., Gussin, G. & Echols, H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2249-2253 (1976).
7. Takeda, Y., Folkmanis, A. & Echols, H. *J. biol. Chem.* **252**, 6177-6183 (1977).
8. Ptashne, M. in *The Bacteriophage Lambda* (ed. Hershey, A. D.), 221-237 (Cold Spring Harbor Press, New York, 1971).
9. Folkmanis, A., Maltzman, W., Mellon, P., Skalka, A. & Echols, H. *Virology* (in the press).
10. Edman, P. & Henschen, A. in *Protein Sequence Determination* 2nd edn. (ed. Needleman, S. B.) 232-279 (Springer, New York, 1975).
11. Roberts, T. M., Shimatake, H., Brady, C. & Rosenberg, M. *Nature* (in the press).
12. Chou, P. Y. & Fasman, G. D. *Biochemistry* **13**, 222-245 (1974).
13. Fasman, G. D., Chou, P. Y. & Alder, A. J. in *The Molecular Biology of the Mammalian Genetic Apparatus* (ed. Ts'o, P. O. P.) **45** (North Holland, New York, 1977).
14. Sauer, R. T. & Anderegg, R. *Biochemistry* (in the press).
15. Ptashne, M. *et al. Science* **194**, 156-161 (1976).
16. Iwai, K., Hayashi, H. & Ishikawa, K. *J. Biochem.* **72**, 359-367 (1972).
17. Pisano, J. J. & Bronzert, T. J. *J. biol. Chem.* **244**, 5597-5607 (1969).
18. Zimmerman, C. L. & Pisano, J. J. *Biochem. biophys. Res. Commun.* **55**, 1220-1224 (1973).
19. Smithies, O. *et al. Biochemistry* **10**, 4912-4921 (1971).
20. Ambler, R. P. *Meth. Enzym.* **11**, 436-444 (1967).
21. Inman, J. K., Hannon, J. E. & Appella, E. *Biochem. biophys. Res. Commun.* **46**, 2075-2081 (1972).
22. Foster, J. A., Bruenger, E., Hu, C. L., Albertson, K. & Franzblau, C. *Biochem. biophys. Res. Commun.* **53**, 70-74 (1973).

## Active transport of picrate anion through organic liquid membrane

SEVERAL studies<sup>1-3</sup> have examined the concentrative transport<sup>4</sup> of ions through bulk liquid membranes. The energy for the transport in these systems is the pH difference—the chemical potential difference of protons across the membrane. We describe here a redox reaction-driven transference system in which picrate anion, a lipophilic anion, moves not only against its concentration gradient but also against its electrochemical potential gradient through bulk organic solvent.

The apparatus is illustrated in Fig. 1. Two aqueous phases (L, left; R, right) are bridged by a dichloroethane layer (M) containing *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD). The aqueous phase, L, contains an oxidising agent, ferricyanide, and R, a reducing agent, ascorbic acid. Figure 1 shows the typical data where the initial concentrations of picrate in L and R phases are the same ( $10^{-4}$  M). The M phase contains  $10^{-4}$  M TMPD which is not equilibrated with picrate before the experiment. An increment of picrate in R phase signifies its corresponding decrease in the L phase. The concentration of picrate in R phase was found to increase and that in L phase to decrease linearly with time after a



**Fig. 1** Concentration of picrate as a function of time. Initial concentration of picrate was the same in L and R phases ( $10^{-4}$  M). The experimental chamber used was essentially the same as a U-shaped glass tube and is illustrated schematically in the inset. The volumes of the aqueous solutions designated as L and R are 15 ml and that of the organic bulk phase, M, is 40 ml. All three phases were agitated with a pair of glass stirrers at a speed of 180 r.p.m. The concentration of picrate was determined spectrophotometrically. The experiments were performed in a dimmed room to prevent light-induced degradation of TMPD. ○, Time course of changes in picrate concentration in R phase with the bulk phase not pre-equilibrated with picrate before the experiment. The aqueous phase on the L side contains 10 mM potassium ferricyanide and the R side, 10 mM sodium ascorbate: both solutions were buffered at pH 9.4 with 100 mM  $\text{Na}_2\text{B}_4\text{O}_7\cdot\text{HCl}$ . Because ferricyanide interfered with the spectrophotometrical determination of picrate, we determined the concentration of picrate in only R phase. The broken line is the calculated value of picrate concentration in L phase. ●, Data obtained with the organic phase equilibrated with picrate. The method of preparation of the membrane is as follows. A given amount of TMPD·2HCl was dissolved with minimal volume of distilled and deionised water (about 0.5 ml), which was poured into 40 ml of sodium picrate ( $10^{-4}$  M) buffered at pH 9.4 with 100 mM  $\text{Na}_2\text{B}_4\text{O}_7\cdot\text{HCl}$ . The free TMPD and the  $\text{TMPD}_{ox}^+ \cdot \text{picrate}^-$  pair in the solution prepared as above were extracted into 40 ml of dichloroethane, which was washed again with picrate solution ( $10^{-4}$  M, pH 9.4). The organic solution was used as the barrier phase, M after the separation of two phases. Other experimental conditions were virtually unchanged. The rate of transference was independent of pH in the range pH 6.5 to 9.5. The pH was adjusted with phosphate or borate buffers (100 mM). □, Transport with voltage clamp at 0 mV. Other conditions as above.



short lag period, and finally a steady state was reached. Picrate was transferred from L to R phase against its concentration gradient.

The lag period became shorter or was absent when M phase had been equilibrated with picrate before the experiments. The slope of the linear portion was approximately equal to that obtained without prior equilibration. All experiments described below were performed using pre-equilibrated dichloroethane. The method of preparing the bulk phase (M) is described in Fig. 1 legend. Figure 2 shows the rates of picrate transport in conditions of varying concentrations of ferricyanide, ascorbate and TMPD. The rates increase with increase of the concentrations of these agents and reach approximately the same plateau level.

The membrane potential has been shown to be important in transport across membranes<sup>4-6</sup>. In the case shown in Fig. 1, the electromotive force (e.m.f.) was initially 160 mV, rapidly decreasing to reach a steady value of 120 mV with the polarity being positive in R with respect to L phase. The e.m.f. acts in the same direction as the picrate flux and hence it is possible that it might be responsible for part of the observed flux. To examine the role of the membrane potential, we clamped the potential difference between R and L phases at 0 mV by short-circuiting a pair of calomel electrodes inserted in L and R phases<sup>7</sup>. This made no difference to the observed results; that is, the membrane potential does not contribute to the transport of picrate in this system. This implies that picrate is being transported against its electrochemical potential gradient, that is, that the transport of picrate in this system is 'active'<sup>8</sup>.

Our results can be explained by the following mechanism. At the L interface, TMPD is oxidised to a cation<sup>9,10</sup>,  $\text{TMPD}_{\text{ox}}^+$  which serves as a carrier for picrate anion,  $\text{picrate}^-$ . The  $\text{TMPD}_{\text{ox}}^+$  and  $\text{picrate}^-$  ion pair migrates down its concentration gradient within M phase. As the ion pair reaches R, picrate is extracted into aqueous phase by virtue of a discharge of  $\text{TMPD}_{\text{ox}}^+$  due to the reducing agent. The neutral reduced form of  $\text{TMPD}_{\text{red}}$  diffuses back to the interface and the process is repeated. Structures of  $\text{TMPD}_{\text{ox}}^+$  and  $\text{TMPD}_{\text{red}}$  are given in ref. 10. The importance of charging-discharging process of TMPD by the reduction-oxidation reaction is also suggested by the fact that in

acidic solutions, the rate of transference slowed down appreciably (data not shown). This may arise from protonation of reduced TMPD,  $\text{TMPD}_{\text{red}} \text{H}^+$  possibly as a result of the lack of the charging-discharging process. The decrease in L phase and the increase in R phase of a negative charge resulting from the transfer of picrate<sup>-</sup> from L to R are counterbalanced by the production of negative charge of  $[\text{Fe}(\text{CN})_6]^{4-}$  from  $[\text{Fe}(\text{CN})_6]^{3-}$  in L phase and by that of  $\text{H}^+$  associated with the oxidation of ascorbate in R phase, respectively.

The transport of cations<sup>1,3</sup> and amino acids<sup>2</sup> against their concentration gradient by coupling to a movement of the other ions in the opposite direction has been reported. It should be noted that no ion is co-transported or counter-transported in the system described here and that the energy for transferring picrate is supplied directly by the free energy associated with the chemical reaction. The substrate to be transported in this system is not restricted to picrate—any lipophilic anions capable of forming lipid soluble in pairs with  $\text{TMPD}_{\text{ox}}^+$  may be transported. It may eventually be possible to prepare a membrane system in which a specific substance is transported by coupling of a chemical reaction.

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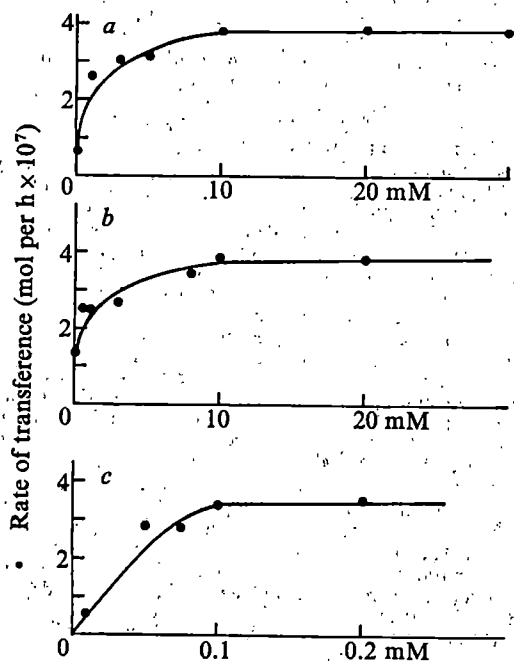
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1. Moore, J. H. & Schechter, R. S. *Nature* **222**, 476-477 (1969).
2. Behr, J.-P. & Lehn, J. M. *J. Am. chem. Soc.* **95**, 6108-6110 (1973).
3. Choy, E. M., Evans, E. F. & Cussler, E. L. *J. Am. chem. Soc.* **96**, 7085-7090 (1974).
4. Harold, F. M. *Bact. Rev.* **36**, 172-230 (1972).
5. Teorell, T. *Prog. Biophys.* **3**, 305-369 (1953).
6. Koefoed-Johnsen, V. & Ussing, H. H. *Acta physiol. scand.* **42**, 298-308 (1958).
7. Ussing, H. H. & Zerahn, K. *Acta physiol. scand.* **23**, 111-127 (1951).
8. Rosenberg, T. *Acta chem. scand.* **2**, 14-33 (1948).
9. Hamill, W. H. in *Radical Ions* (eds Kaiser, E. T. & Kevan, L.) 322 (Wiley-Interscience, New York, 1968).
10. Trebst, A. *Trends biochem. Sci.* **60-62** (1976).

Fig. 2 Rates of transport in conditions of varying concentrations of ascorbate, ferricyanide and TMPD. *a*, Rates plotted against the concentration of ascorbate where the concentrations of ferricyanide and TMPD are kept constant at 10 mM and 0.1 mM, respectively. *b*, Rates against  $[\text{Fe}(\text{CN})_6]^{3-}$  with ascorbate and TMPD kept constant at 10 mM and 0.1 mM, respectively. *c*, Both ascorbate and  $[\text{Fe}(\text{CN})_6]^{3-}$  are held constant at 10 mM. In all these experiments, the pH value is 9.4 buffered with 100 mM  $\text{Na}_2\text{B}_4\text{O}_7\text{-HCl}$ , and the initial concentrations of picrate is  $10^{-4}$  M.



## Erratum

In the article 'Mathematical models for the evolution of multi-gene families by unequal crossing over' by A. S. Perelson & George I. Bell, *Nature* **265**, 304-310 (1977), on p. 304, paragraph 1, line 10, 'randomly' should read 'tandemly'; p. 305, paragraph 6, lines 15 and 16 should be replaced by 'crossover there are three possibilities: (1) the number of copies of gene *i* remains the same, (2) increases by one, or (3) decreases by one'; paragraph 6, lines 17 and 18 ' $[1 - \lambda_n(t) - \mu_{n1}(t)]$ ,  $\lambda_{n1}(t)$  respectively' should read ' $[1 - \lambda_{n1}(t) - \mu_{n1}(t)]$ ,  $\lambda_{n1}(t)$  and  $\mu_{n1}(t)$ , respectively'; p. 306, the right side of equation (3a) should read

$$P \frac{n_i}{N(t)};$$

p. 306, last line in left column, 'variance' should read 'second moment'; p. 306, the summation index should be '*i*' not '*T*' in the equation for  $N(t)$  following equation (3b); p. 308, line 4, should read ' $P_{N_0 \rightarrow 1}(t)$ ' not ' $P_{N \rightarrow 1}(t)$ '; equations (12) and (13), '*P*' should read '*P*<sub>0</sub>'; the left side of equation (15) should read

$$p_i(t) = \int_0^t \frac{dp_i(t')}{dt'} dt'$$

the left side of equation (16) should read

$$\int_0^t t' \frac{dp_i(t')}{dt'} dt'$$

paragraph 4, line 18, 'context<sup>38</sup>' should read 'context<sup>39</sup>'; p. 309, equation (18) ' $1 \leq \xi_1 \leq m$ ' should read ' $0 \leq \xi_1 N_0 \leq m$ '; equation (19) ' $0 \leq \xi_2 \leq m$ ' should read ' $0 \leq \xi_2 N_0 \leq m$ '; equation (20), the summation indices ' $\xi_1$ ' and ' $\xi_2$ ' should read ' $\xi_1 N_0$ ' and ' $\xi_2 N_0$ '.

# reviews

## Immunological sourcebook

Elizabeth Simpson

*Lymphocyte Differentiation, Recognition and Regulation.* By David H. Katz. Pp. xiii+749. (Academic: London and New York, 1977.) \$42; £29.80.

THE scope of this book is no less than a review of the whole of current basic immunology. This is approached under a series of chapter headings dealing with the surface antigens of T and B cells and their respective ontogenies; receptors and specificity of these two major subclasses and their functional properties; regulatory interactions in immune responses, including genetic control and the role of the major histocompatibility complex; and finally, immunological tolerance.

This is a monumental assignment for one person to undertake. Two thousand three hundred references are quoted, most of them published since 1970. Dr Katz's approach has been to consider his selection of the relevant references under the headings he has chosen, in many cases quoting substantial details of the work under discussion. In chapters where he has integrated the results of several papers and the views of different authors, the story emerges as an interesting one, and his own views are illuminating. This successful approach is seen most in the chapters dealing with T-B cell cooperation, a field in which the author has been extensively engaged. One can argue with him as to his choice of relevant references, and the reservations, many times reiterated, with which he regards data gathered from *in vitro* systems, but the overall synthesis of these chapters is good; they are both informative and provocative.

The chapters dealing with subjects outside the author's own experimental field suffer from repetition and a lack of synthesis of the data and in the discussion presented. Some sections read like a catalogue of papers; for example, the section on H-2-restricted cytotoxic responses describes sequentially and in great detail each of the examples (this class of response to virus, hapten and minor transplantation antigens), and fails to underline the essential similarity of the systems and

the important biological implications of the findings. The general lack of cross-referencing, either in the text, or by way of the index (which is totally inadequate) is most noticeable and irritating in chapters which proceed by cataloguing rather than by synthesis. For example, the question of the biological importance of polymorphism in the major histocompatibility complex (MHC) comes up in the chapter on T and B lymphocyte surface antigens in the sub-section on biochemistry of MHC antigens (p78), in the chapter on "Functional Properties of T Lymphocytes" during consideration of H-2-restricted cytotoxic responses (pp 281-296) and in the chapter on genetic control of immune responses (p530 *et seq.*); and yet there is no cross-referencing of the discussions therein.

The organisation of the chapters under the chosen headings has led to some loss of clarity. This is particularly evident in the chapter entitled "Functional Properties of T Lymphocytes" subdivided into (A) "Regulatory T Lymphocytes" and (B) "Effector T lymphocytes". Such a classification, which quite arbitrarily considers helper and suppressor T lymphocytes under the first heading and mixed lymphocyte reaction proliferative T cells, cytotoxic T lymphocytes and T cells mediating delayed type hypersensitivity under the second, obscures the distinction between precursor T cells for any particular function and effector cells which have differentiated as the result of exposure to antigen. The distinctive phenotype of the precursor cells for each function has been documented from many laboratories using anti-Ly antiserum, and implies a precommitment to function before encounter with antigen. This data on precommitment is contained in several of the references quoted in the bibliography but Dr Katz makes only brief mention of the notion, quoting just one of the references in a manner suggesting that it contains the only data available on the point (p265).

In a different context Ly phenotyping is mentioned under the heading of "Surface Phenotype Antigens" (chapter VIII) and in the chapter on

"Surface Markers of T Lymphocytes" (chapter II). Discussion of the data, however, is scattered in the text, and these are not used to integrate earlier, inevitably less well defined observations on the separation of functional subsets of T cells. In some cases, as in the chapter on "Ontogeny of T Cells", they are not mentioned at all.

Chapters X, on "Regulatory Cellular Interactions", XII on "Genetic Control of Immune Responses" and XIII on "Mechanisms of Regulatory Cellular Interactions" obviously contain overlapping material. In some cases, the division chosen has fragmented and obscured the story; for example, the failure to follow through the discussion on T-B cooperation in chapter X with the data on factors, which is presented in chapter XIII. The factor chapter (XIII) itself is incomplete in as much that the genetically restricted factor of Erb and Feldmann is mentioned not here, but in the previous chapter, to which there is no cross-reference on this point, and the suppressor factor of Kontiainen and Feldmann is not mentioned at all.

There are some misquotations of references, some by omission (that is, failing to take account of the whole of the contents of a paper, in some cases, stating that the author(s) had not looked at a particular point, when they had done so) and some by addition (that is, fanciful addition to the data); but this must be almost inevitable when so many references are considered, and does not occur often enough to mar the value of the text and its very extensive bibliography for serious readers. Informed immunologists will be able to seek out the discussion and references they want by assiduous detective work which would not have been necessary had the index been adequate and the cross-referencing in the text more thorough. For less informed readers—for example, graduate students—this valuable sourcebook and bibliography will not be so accessible. □

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## Nutrition and the brain

*Nutrition and the Brain*. Vol. 1: Determinants of the Availability of Nutrients to the Brain. Pp. xi+324. Vol. 2: Control of Feeding Behaviour and Biology of the Brain in Protein-Calorie Malnutrition. Edited by Richard J. Wurtman and Judith J. Wurtman. Pp. 313 (Raven: New York, 1977.) £19.50 each volume.

A NUMBER of years ago a distinguished neurochemist remarked that if people continued to take up neurochemistry at the rate obtaining at the time, then everyone in the world would be a neurochemist long before the end of the millennium. The rate at which books about nutrition and the brain have recently appeared seems to threaten a comparable natural disaster; and here are two more. The fact that your reviewer seems to be one of the few workers in the field who has not yet found the energy to produce one, may be the reason for a certain weariness, if not sourness in his approach and a developing propensity for denunciatory overstatement. But at least it will be agreed that a field with so much contemporary competition demands more than usual justification from the late arrivals.

Someone, somewhere, will find almost all of them very useful; but unfortunately the very misleading title of the series, *Nutrition and the Brain*, will not lead the literature surveyor to many of the better chapters unless they appear separately in the indexes under such headings as 'Cerebral Energy Metabolism', 'The Natural Diet of Primates', and so forth. However widely one interprets the word 'Nutrition', and however all-pervasively 'the Brain' controls the rest of physiology, the Editors have surely assumed too readily that their subject encompasses the whole of nutritional biochemistry and neurobiology.

Thus, if the young scientist entering the field happens to be fascinated by evolutionary aspects of the natural diet of Primates, then he will be as entranced as was your reviewer by the erudition of the first long chapter. But who is to blame him if he reflects that all the mammalian orders grow remarkably similar brains (in compositional and metabolic terms at least) whether they are gatherers or hunters, carnivores or vegetarians, ruminants or rodents; and that these matters are therefore unlikely to concern him. If he is a neurochemist, he will already have read one of Sokoloff's other excellent accounts of cerebral energy metabolism. If he is not, I suspect he

will not be much helped, even by such a classic account, until we reassure him that the subject is not one which bears at all directly on the problems of the starving billions or overstuffed millions of this wicked world.

The chapter on amino acid availability to the brain may at first sight seem more relevant, especially to those who have not yet caught up with the 'protein fiasco' and still believe that what the Third World needs most of all from us is steak. The whole topic of the blood-tissue relationships of the brain with regard to amino acids is a marvellous one for those interested in transport mechanisms or in certain in-born errors of metabolism. It is, however, of no direct interest in the present context.

It has been known for some time that certain putative neurotransmitters are synthesised within the brain from particular precursor amino acids; that the blood levels of these latter influence the brain levels of the former and are themselves influenced by dietary intake. It therefore persuasively follows that in this particular instance, something about the diet influences substances which are held to be to do with sleep, or mood, or otherwise with neurotransmission. This leads by delightful prestidigitation to the argument that since, under *in vivo* conditions, one metabolic pathway in the brain can be shown to be controlled by precursor substrate concentration; and since the latter is often derived from dietary sources, then who knows but that all the other metabolic pathways may not ultimately be under nutritional control as well? We can therefore discuss as many of them as we please. I suppose it depends what you mean by "ultimately". If this kind of argument persuades you of the relevance to 'Nutrition and the Brain' of its energy metabolism; of amino acid, folic acid, B<sub>12</sub> and choline availability to the tissue, and of the role of B vitamins in nervous function, then these books are for you; but not, I fear, for me.

You may even be interested in the blood-brain barrier, other than as a convenient incantation for writing off phenomena which are otherwise difficult to "explain". In that case you can choose between chapter 2 in which the BBB is responsible for the brain's metabolic idiosyncracies but whose nature and location are uncertain; and chapter 3, in which the anatomical site is no mystery to the author and its properties quite well enough defined to "explain" a hopelessly mixed bag of transport mechanisms; and chapter IV which gives us yet another point of view on what may be the best-preserved neurobiological hoax of the twentieth century. At least the impartial reader

will not be lulled into complacency by any uniform consensus, and the hunt will continue for some kind of impermeable wash-leather separating the blood from the brain.

Volume 2 gets prematurely bogged down in a discussion of the control of eating behaviour in 145 pages with 827 references, as if the main elements of the main title (*Nutrition and the Brain*) have in some way become juxtaposed. The remaining three chapters, however, at last get down to the matter in hand. A chapter on the effects of undernutrition on brain morphology is mostly, and properly, about influences on brain development, although the authors complain that there is no information about the adult state in this respect. Could it not be that there is nothing interesting to see in the brains of starved adults (except in those exceptionally rare encephalopathies so beloved of the last chapters in tomes on clinical neurology)? I happen to think that in this whole, surprisingly lesion-free neuropathology of developmental undernutrition, the most rewarding contemporary growing-point may lie in current attempts at quantitative neurohistology. So, it is a bit disconcerting to be quoted here as not thinking much of the idea. There are some formidable technical obstacles to be overcome, however, before brain structures can be realistically counted, so that the whole of world knowledge in this area could be summarised on half a page with about four references; thus, it is perhaps to be expected that this chapter too dwells on matters outside its stated subject, or fills in the pages by reproducing chunks from elementary textbooks of neurohistology.

The second half of volume 2, however, blossoms forth with two quite outstanding chapters on the effects of widespread varieties of malnutrition on biochemical aspects of brain development, and on human behaviour. Both are masterpieces of clarity: the first in systematically describing what we know without ambiguity; the last in making it perfectly clear that there is no more difficult and confusing topic than the effects of infant malnutrition on human behaviour. The first, by Nowak and Munroe will be a classic reference point for a long time to come. The last, by Pollitt and Thomson should be a powerful antidote to those politicians and the less perceptive of their scientific advisers who still persist in that simplistic non-question, "Does infant malnutrition cause mental retardation?"

John Dobbing

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## Low temperature physics

*The Quest for Absolute Zero: The Meaning of Low Temperature Physics.* Second edition with SI Units. By K. Mendelssohn. Pp. 281. (Taylor and Francis: London, 1977.) £5

Low temperature physics, for those who know little of it, often seems to represent the very epitome of scientific futility: a seemingly purposeless (and highly expensive) competition to see who can approach most closely the unattainable absolute zero of temperature. They should read this book. It would be very likely to change their minds since, as an apologia for one of the most fascinating and fruitful areas of physics to emerge this century, it could hardly be bettered.

Mendelssohn sets low temperature physics squarely in its historical and scientific context, close to the centre of the stage during that quite unprecedented explosion of knowledge which took place in physics in the early decades of the present century. Using an entirely non-mathematical approach, he explains with impressive clarity and accuracy how many of the fundamental concepts of modern physics arose from, or were illuminated and supported by, experiments on the novel states of matter which exist at very low temperatures.

The first four chapters are largely historical in structure, the story opening with Cailletet's liquefaction of oxygen (90K) in 1877, running on to describe the liquefaction of hydrogen (20K) by Dewar in 1898. This first section ends with an assessment of the achievements of Kamerlingh Onnes who, by liquefying helium (4K) in 1908 and discovering superconductivity, became the father of low temperature physics as we know it today. This section includes explanations of the isotherms of real (non-ideal) gases, critical points, cascade liquefiers and expansion engines.

Unusually, Mendelssohn considers how the personal attributes of the researchers may crucially have influenced the extent of their scientific achievements. For example, Dewar, portrayed as a brilliant but autocratic and quarrelsome loner, failed in his attempt to liquefy helium. The prize fell, instead, to the much more open, equable and diplomatic Onnes who possessed the inclination and ability to create a scientific laboratory in the modern sense, successfully coordinating the activities of a comparatively large complement of technical staff, colleagues, and visitors from other universities.

The next five chapters include vigorous and readily understandable accounts of topics such as the Third Law, heat, entropy, zero point energy, low temperature specific heats, quantisation, radiation, indeterminacy, gas degeneracy, quantum statistics of ideal gases, Fermi surfaces, Curie's Law, magnetic cooling and the various ramifications of superconductivity. Individual contributions to the developing conceptual structure made by such figures as Bohr, Born, de Broglie, Einstein, Fermi, Heisenberg, Nernst and Schrodinger, many of whom were known personally to the author, are carefully described and assessed.

At this point, the second edition diverges from the original, published in 1966. A whole chapter is now devoted to cryogenic technology, dealing with SQUIDS (superconducting quantum interference devices, used for measuring tiny magnetic fields), computer elements, superconducting motors and generators and, of course, superconducting magnets. The final chapter, on superfluidity, has also been extensively reworked to take account of recent advances, particularly in the new and rapidly growing field of superfluid helium-3. Even so, the pace of current cryogenic development seems

to have outstripped production of the book which, for instance, describes dilution refrigeration as capable of "temperatures approaching 0.01K in continuous operation", whereas 0.003K is now routinely being reached by this technique at Grenoble.

The coloured line drawings are helpful. There are also a number of photographs portraying most of the major scientific figures discussed in the text: of particular note is one entitled: "The heyday of low temperature physics. L. D. Landau in discussion with the author in Moscow, 1957."

As might be expected in a work with this breadth of vision, containing so much of the author's personal viewpoint of the scientific world, pungently and entertainingly expressed, there is much with which practitioners may take issue; but this in no way detracts from the success of the book as a whole. Like the previous edition, it can confidently be recommended to anyone who would like to know more about the behaviour of matter in that unimaginable abyss of coldness around and below 1K.

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## Mammalian development

*Developments in Mammals.* Vol. 2. Edited by Martin H. Johnson. Pp. 241. (North-Holland: Amsterdam, New York and Oxford, 1977.) Dfl. 72; \$29.50.

THE second volume in this interesting and timely series continues in the style and format established in the first. There are both good and bad aspects to this. It was hoped that the small and inadequate typeface would have been improved but this volume is also difficult and tiring to read. Such is the speed with which these two volumes have been produced that the changes in typeface could not be introduced in volume 2. I for one look forward to their introduction in the next issue.

The good balanced mixture of developmental biology, reproductive physiology and biochemistry has been maintained. Two papers concern growth and maturation of oocytes, and transport and selection of sperm. These are followed by three papers each considering the cleavage stages of rodent development from slightly different viewpoints. This section is particularly welcome as the subject is somewhat controversial, with much circumstantial evidence concerning determination and differentiation of the two cell lines which emerge during cleavage. Here, in these three papers, all the

evidence is reviewed, and the various interpretations that have been put on the data are well discussed. Of the remaining four papers, one reviews the *in vitro* culture systems and their application to the peri-implantation period, whereas the other three address another hotly controversial subject—the involvement of steroid hormones in controlling implantation and in particular to what extent the mammalian blastocyst may produce steroids and thus perhaps control, at least in part, its own implantation.

Encouraged by editorial policy, authors have occasionally been speculative and have also used the book as an outlet for previously unpublished data. Although this policy has obvious advantages in publications such as this series, there are hazards since papers are edited rather than refereed, and somewhat one-sided arguments occasionally slip through the net. In this second volume, the editor has provided the best possible answer to this problem by gathering together several papers on one subject, thus presenting argument and counter-argument side by side. For scientists new to the field, this is especially useful. Although expensive, this volume is good value and augurs well for the next of the series.

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## Shallow layers of the Continental Shelf

*Exploring the Geology of Shelf Seas.* By R. McQuillin and D. A. Ards. Pp. 234. (Graham and Trotman: London, 1977.) £10.50.

As with many books, the title is a little misleading. Although the subject matter is concerned with general techniques that may be used in mineral or even oil exploration, it would be incorrect to say that the book is concerned with exploration specifically. The NERC/IGS, of which the authors are members, uses the techniques described more as mapping devices than as exploration tools.

The purpose of the work is to describe how information about the shallow layers of the Continental Shelf down to about 300 metres or beneath Quaternary cover, may be obtained. Over the past decade, considerable attention has been given by the oil companies to the "deep geology" of the North-West European Continental Shelf resulting in the discovery of major oil and gas fields in almost every geological formation from the Carboniferous to the Tertiary. In contrast, however, very little is known of the near subsurface, and there are hardly any maps or data published on this topic, even by the IGS who have by their own confession done the most work. There is thus a large gap in our knowledge about the nature and extent of bottom sediments. This is the region with which the IGS is concerned and on which the book concentrates.

Seven of the chapters are devoted to geophysical techniques and only one to the acquisition of geological information by sampling and drilling. This tends to give the book a basic imbalance, and it is often not fully clear how the results of geophysical work are utilised by the geologists in making maps. Just such an account would have placed the book in perspective. Nonetheless, the book is clearly written and the reader should have little difficulty in understanding basic principles even if he is left a little in doubt as to the usage of the methods.

The opening chapter deals with the problems of position fixing at sea. This is a useful chapter by itself, as it is often difficult to find concise accounts explaining the various methods. Once at sea, two sorts of observation may be made. The first area is the sea itself, and the nature of the seafloor; the second, the strata beneath the sea bed, from which information may be

obtained either directly or indirectly. Ways of studying the former using echo-sounding and side-scanning solar techniques thus follow, but by far the greatest emphasis is placed not unnaturally on the latter: gravity and magnetics, but particularly continuous seismic profiling techniques, both sub-bottom-sparker—and deep penetration seismic. These tools, perhaps above any others, have provided the means of mapping large areas using relatively few geological control points.

Although well intended, the chapter on sampling methods does not fully relate to the rest of the book, and is itself somewhat disjointed, reading

## Planetary atmospheres

*The Physics of Atmospheres.* By J. T. Houghton. Pp. xiii+203. (Cambridge University: Cambridge, London, New York and Melbourne, 1977.) £6.50.

PROFESSOR HOUGHTON has written a most interesting book with the intent "to introduce physics students at both undergraduate and graduate levels to the physical processes which govern the structure and circulation of a planetary atmosphere." He is completely successful. The book is very strong on the aspects of radiative transfer, as might be expected because Houghton's group is the world leader in this field. Chapter 2, "A Radiative Equilibrium Model", and chapter 4, "More Complex Radiation Transfer", are completely devoted to radiative transfer; the latter includes a most valuable discussion of how to proceed from molecular properties, such as line widths, to computations of radiative heating rates in realistic atmospheres. Chapter 5, "The Upper Atmosphere", also focuses on radiative transfer and contains a unique detailed treatment of the breakdown of thermodynamic equilibrium. Chapter 6, "Clouds", is mainly concerned with their radiative properties.

Chapter 7, "Dynamics", chapter 8, "Atmospheric Waves", and chapter 10, "The General Circulation", provide an excellent introduction to the role of the large scale motions in governing atmospheric structure. I found the approach here, and in chapter 9, "Turbulence", straightforward and efficient. It reminded me of some very clear lectures on the subject that I heard from Dr John Green of Imperial College in 1971. Houghton acknowledges a set of dynamics' lectures by Dr R. S. Harwood who was formerly associated with Dr Green. From these chapters, the student could easily tackle the more advanced texts on atmospheric dynamics.

Chapter 3, "Thermodynamics", in-

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more like a catalogue than an explanation of how geophysical data is used to site sampling and drilling locations, or indeed of how maps are constructed.

One cannot hope, however, for everything, and the book will be of value in explaining little-known facets of geophysical techniques to students of the earth sciences who might otherwise find it difficult to locate the information.

John Brooks

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cludes a treatment of available potential energy as crystallised by Lorenz. This concept is carried, too, in later chapters and is most valuable in explaining structure. I have used it myself in lectures on the physics of the upper atmosphere. One can most easily perceive from this approach that the observed structure is the result of a continuous interplay between radiative and dynamical processes.

Chapter 11, "Numerical Modelling", and chapter 12, "Global Observation", again stress the radiative transfer aspects. Chapter 13 has the longest title of all, "Atmospheric Predictability and Climatic Change", but consists of only two pages of text and three figures, and seems to be an unnecessary afterthought which is not at the same level as the rest of the book. Chapter 6 is also a little too short to catch the essence of the physical aspects of clouds. These are, however, minor complaints, for the book as a whole is very well written, and does not pretend to provide all of the physics of atmospheres.

I learned much from working through some of the examples (provided with solutions) which are an integral part of the text. The interested student could, in fact, teach the subject to himself with the text, the useful information in the appendices and a hand-held calculator. Although references are provided, little from them is needed to work through the text. I recommend the book to all physics students interested in atmospheres. To all physics students, the text presents live examples of the laws of physics, which may replace the older examples of steam engines and ice skaters. Many first-year meteorology students would also benefit from working through this text. An early overview of the subject might be very helpful before immersion in two years of concentrated courses at the graduate level.

Reginald E. Newell

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## Quirks and strong points

*Psychologists on Psychology.* By David Cohen. Pp.360. (Routledge and Kegan Paul: London and Henley, 1977.) £6.95.

PSYCHOLOGY nowadays is a very mixed bag, and indeed some would query whether it is really just one bag. David Cohen's book, which is a series of interviews with successful psychologists, illustrates very well how far apart from one another they are. It is not so much that they disagree, though disagreements there are in plenty in this enterprising book. What is much more striking is that the different psychologists featured in this book are often so far apart in their subject matter, their approach, their conceptual frameworks, and their aims, that even mutual criticism is out of the question.

The interviews which David Cohen

gave to these stars of the behavioural world seem to have been well planned, and the questions are searching enough to bring out the subject's quirks as well as their strong points. Perhaps Cohen should have been more critical but even here there is an advantage of some interest, because the calmness of the interviews seems to have brought out into the open a staggering complacency in some of the psychologists concerned.

David Cohen's attempts at the beginning and end of the book to find some link between the backgrounds and personalities of the different interviewees—a psychology of psychology—are almost certainly ingenuous; these people are as diverse as their work. The interviews which form the core of this book, however, give a clear, conveniently quick though often disquieting picture of what many of the present leaders of psychology think they are up to.

**P. E. Bryant**

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## Androgen action

*The Mechanism of Action of Androgens.* By W. I. P. Mainwaring. Pp. x+178. (Springer: New York and Berlin, 1977.) DM65.40; \$28.80.

THIS is a useful monograph written by an acknowledged expert in the field of androgen action. The book is an attempt to describe the molecular events associated with the entry of testosterone into androgen-sensitive cells and the subsequent metabolic changes associated with the presence of the hormone in these cells.

The author uses a temporal approach to the problem of the analysis of androgen action and, as usual, much of the material is drawn from experiments on the rat ventral prostate. The author is careful to point out the reservations which must be applied in generalising from this animal model to any other species or tissue.

The core of the book is devoted to an account of the initial events and the early and late events associated with androgen stimulation of target tissues. The author points out that, although androgens influence transcription and the synthesis of RNA, they may also affect other processes. The manner in which androgen metabolism in the target tissues influences the mode of action of androgens is considered in detail. The high affinity binding sites,

the intracellular transformation and the subsequent nuclear retention of the hormone are reviewed. The need to isolate and characterise the androgen receptor proteins is stressed. It is a pity that the author did not devote more attention to the effects of androgens on muscle and perhaps develop a working hypothesis or speculate on how they may affect this large body mass.

In the latter part of the book, the author critically reviews his own studies showing that some of the actions of androgens are explicable on the basis of the increased production of selected mRNAs and ribosomes. Thus, androgen action is at least in part an enhancement of genetic transcription.

The author writes: "The halcyon days of studies on the mechanism of action of androgen are drawing to a close." In fact, this excellent text is a clear indication that this is not so. Future research on the mode of action of androgens may, however, require more sophisticated approaches and more elegant tools. The book is clearly written and the reader gets the impression of being led through some of the mysteries of androgen action as in a series of lectures. The text has the stamp of authority. It is written by a scientist who has in fact uncovered many of the interesting facts on the mode of action of androgens. The book will be valuable to researchers and to university teachers concerned with the mode of action of steroid hormones in general and androgens in particular.

**G. S. Boyd**

*G. S. Boyd is Professor of Biochemistry at the University of Edinburgh, UK.*

## Geomorphology and remote sensing

*Remote Sensing in Geomorphology.* By H. Th. Verstappen. Pp. x+214. (Elsevier Scientific: Amsterdam, New York and Oxford, 1977.) Dfl.98; \$39.95.

THIS well produced book will be valuable to the postgraduate or advanced research worker, rather than as an undergraduate textbook. A large amount of material is covered in a somewhat condensed style. Perhaps one of the chief merits of the book lies in its extensive bibliography of sources in English, German, Dutch, Russian and French languages. Of the 214 pages, approximately eleven are close printed lists of references, although some sources seem to have too brief a description, which may not be sufficient for procurement.

The pages are a source of constant stimulus: a technique or idea is introduced, and briefly described, but the reader will have to go to the original source for full details. This is perhaps a natural result of a text from an authority with the length and breadth of experience of the author.

The illustrations are plentiful and well produced. Some are in colour, and a notable feature is the number of stereopairs printed side by side for viewing with a pocket stereoscope.

Subjects covered include an historical introduction, a description of the types of imagery available, and available methods of interpretation. Chapters are devoted to image analysis based on relief (stereoscopy) and density criteria; pattern and texture are subsumed within these headings.

Geomorphology itself is divided into genetic and environment divisions, the latter including treatment of land systems methods of survey. This means that environments and major zones such as coastal, glacial, tropical and fluvial are discussed using examples hard upon one another's heels, with photography, radar, thermography and satellite imagery also intermingled. The examples used include lunar and planetary geomorphology. A final chapter deals briefly with such vital items as relative costs and fieldwork methods.

This is a most stimulating book, but one to be dipped into and used for reference or browsing. Because of the condensed staccato style, however, it is somewhat indigestible.

**J. R. Hardy**

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● In the review of *Perpetual Motion: The History of an Obsession* (*Nature*, 20 October, 269, 731; 1977), the price should have read £5.50.

## Biochemistry and pharmacology of venoms

*Venoms: Chemistry and Molecular Biology.* By Anthony T. Tu. Pp. 560. (Wiley-Interscience: London and New York, 1977.) \$43; £25.90.

VENOMS have always had a peculiar fascination. How do they kill animals (and, in particular, humans)? What do they contain, and can any of the components be put to use either as tools in research or as therapeutic agents? Before the advent of modern chromatographic techniques, not much progress could be made in the field but since then there has been (to say the least of it) no shortage of papers. The quality of some of these has, however, been exceedingly bad. Separation procedures still require some skill and if the skill is lacking they lead to unspecified mixtures. The desire to inject such mixtures (or, indeed, whole venoms) into animals and see what happens is (apparently) strong. It ought always to be firmly resisted.

All of this means that to write a book on venoms is a formidable undertaking. It needs a considerable knowledge of the relevant aspects of chemistry, biochemistry and pharmacology and, if one is to deal with the effects of venoms in humans, of clinical medicine as well. Enough to daunt the strongest spirit. Professor Tu admits this in his preface and uses the phrase "monumental task". He has, in the event, produced a book containing over four hundred pages devoted to snake venoms (his own research interest) and rather less than one hundred to other animal venoms (scorpions, bees, wasps, and so on). One could hold the view that this is a trifle unbalanced, since (for example) the components of bee venom have been extensively investigated both chemically and pharmacologically. On the other hand, it is true that a bewildering variety of peptides and proteins have been found in snake venoms and some of these have proved to be extremely useful. For example, the phosphodiesterase contained in many snake venoms is an invaluable tool in the determination of nucleotide sequences in nucleic acids. On these grounds, the long section of the book on the enzymes present in snake venoms can, no doubt, be justified.

I must confess, however, that I found the book to be essentially uncritical. Most of the papers quoted seem to be given equal weight when clearly some of them (all too many, I fear) are un-

satisfactory on chemical or biological grounds or both. What, for example, is the point in giving a list (p509) of the values reported for the (so-called) LD<sub>50</sub> of apamine in mice when it is perfectly clear that the correct value is that given by Habermann (in 1972, incidentally, and not as quoted) and subsequently confirmed by other workers? How does one feel about the (supposed) component of bee venom called "cardiopep"? Ought one not to entertain the suspicion that the biological effects ascribed to it are really due to noradrenaline (shown to be present in the venom, as distinct from the venom gland, by Banks *et al.*, 1976, and not, as given, by Owen, 1971)? Again, what about minimine? This is certainly my favourite substance (or non-substance) because its biological activity is supposed to be the produc-

tion of miniature flies from the larvae of *Drosophila melanogaster* (whoever would think of such an experiment?) One could continue, but the list of doubtful substances and even more doubtful biological activities would be long and tedious. In defence of Professor Tu, I must say that, in my view, no one man could read and assess critically all the literature in this area.

It is a pleasure to be able to say that the book is well produced, well laid out, and that the references are remarkably up-to-date and (for the most part) remarkably complete. The book will certainly be of value (if only for the references) to workers in the field and to those wishing to enter it.

C. A. Vernon

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## Basic statistical handbook

*A Handbook of Numerical and Statistical Techniques.* By J. H. Pollard. Pp. xvi + 349. (Cambridge University: Cambridge, London and New York; 1977.) £13.50.

WHEN books on basic statistics continue to appear with amazing regularity, it is refreshing to find one which more than permutes the 'usual material'. This book not only describes essential statistical techniques but gives a welcome priority to elementary numerical methods.

The author aims to help computer users, firstly to understand the output of statistical packages, and secondly to adapt these and similar packages for programmable calculators to suit their own requirements. Although it is not a textbook of elementary numerical and statistical techniques, its uniformity and clarity of presentation, together with its comprehensiveness, should give it wide appeal. A basic knowledge of mathematics is assumed.

The contents have been skilfully divided into three parts. Part I (seven chapters), after a brief introduction to essential basic mathematics and a helpful discussion of sources of error, describes numerical methods for locating the roots of non-linear equations, data smoothing, integration, differentiation and interpolation. It finishes with a variety of useful techniques including the regrouping of grouped data, the fusing of smooth curves and steepest descent.

Basic statistical techniques, except regression, are presented in part II (seven chapters) which, after briefly

introducing probability theory, describes the important frequency distributions including the Pearson system. Two chapters devoted to hypothesis testing and point and interval estimation provide a broad survey of parametric and distribution-free statistical tests. Each test is presented in a standard format which facilitates both comprehension and correct application. This part ends with three special topics—random numbers, data transformation, and censored and truncated distributions.

Part III (four chapters), devoted to the method of least squares, describes simple linear regression (with and without matrix notation) and most of the associated statistical tests. (Unfortunately, the statistical comparison of two or more regression lines has been omitted.) Curvilinear and multiple linear regression are presented succinctly, using matrix notation, and this part ends with an introduction to non-linear regression.

Throughout the book, each technique is separately referenced and well illustrated with a fully-worked example, usually from the life sciences. Specialised tables are sensibly interspaced with the text, while those of more general application are placed in an appendix. Every chapter includes a selection of exercises though unfortunately the answers are not given.

This handbook has been carefully assembled, it is easy to use, and cross-referencing is kept to a minimum. It should be a valuable asset to the scientist and the statistician.

A. L. Johnson

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# obituary

## Bruce C. Heezen

PROFESSOR Bruce C. Heezen of the Lamont-Doherty Geological Observatory, New York, died on 21 June 1977 at the age of 53 of a heart attack while working on board the U.S. nuclear research submarine NR-1 studying the Reykjanes Ridge south of Iceland.

With his late colleague, Professor Maurice Ewing who was founder and director of the Lamont (later Lamont-Doherty) Geological Observatory, Bruce Heezen played a major part in the rapid post-war expansion of our understanding of the morphology and geology of the deep ocean floor, on which the now widely accepted theory of plate tectonics is based.

During the war the techniques for studying the deep ocean improved immensely so that in the early 1950s all sorts of new geological and geophysical data were being collected from the increasing number of research cruises. With Maurice Ewing, Bruce Heezen saw the enormous potential in the thorough compilation and analysis of these data, especially the morphology, on a world-wide scale. Because of the restrictive attitudes of the U.S. Navy on the publication of contour charts, he was forced to develop, with the cartographic skills of Marie Tharp, the physiographic diagram style of presentation of seabed morphology. These charts, several of which were published by the National Geographic Magazine, have been widely used by those interested in the oceans and have contributed significantly to an appreciation of what lies beneath the sea surface. His analysis of ocean floor morphology, initially of the North Atlantic and published as a Special Paper of the Geological Society of America in 1959 but later extended world-wide, became the basis of a physiographic classification used now by oceanographers, geologists and even by lawyers.

During these morphological studies in the late 1950s he made what I believe to be his greatest contribution to the earth sciences. He recognised that the major ridge systems which lay roughly in the centre of many oceans were in fact linked into one continuous, although sinuous, mountain range 40,000 miles long and covering an area equal to that of all the continents combined. He found that the axis of this mid-ocean ridge system was associated with shallow seismicity and was able to predict the existence of hitherto unknown ridges southeast

of Africa and across the Arctic ocean. Along the axis Heezen, and his colleagues Tharp and Ewing, noted a more or less continuous valley which was morphologically similar to and actually linked to the Red Sea and the East African rifts, and also to the tension cracks in the central Icelandic graben. It was clear that along this 40,000 mile axis the crust of the earth was under tension and splitting apart. Heezen saw this initially as evidence for an expanding earth but dropped this idea when the subduction mechanism was discovered to absorb the excess crust and global plate tectonic theory developed.

Although Heezen was able to view the oceans on a global scale, he was concerned with all scales of deep-sea geological processes. He recognised that far from being quiet passive regions, the abyssal deeps were disturbed by fierce and destructive turbidity currents which contributed to the cutting of submarine canyons, transported sediment great distances along the sea floor and which gave rise to abyssal plains. He was interested in all modes of sedimentation and pioneered the study of deep-sea bedforms, such as sand waves and dunes, and their relation to near-bottom ocean water movement. In his search for details of bottom processes he exploited the use of bottom photography to the full, superbly and readably presented to the general public in his book with Hollister, *The Face of the Deep* (1971) nominated for a U.S. National Book Award, and in the last decade he made numerous dives in research submersibles to observe the bottom directly.

Heezen was much concerned with the practical problems presented by the deep ocean. For years he advised the cable industry on the potential hazards to deep-sea cables from geological causes and much of his submersible work was directly related to naval requirements. He was an ardent supporter of international collaboration, especially the GEBCO bathymetric chart project, and pioneered the cartographic presentation of geological and tectonic data for the Commission for the Geological Map of the World.

Born in Iowa, Bruce Heezen obtained his bachelor's degree from the University of Iowa in 1948 and his Ph.D. from Columbia University in 1957. He joined Maurice Ewing in the formative stages of the Lamont Geological Observatory and spent his work-

ing life there, becoming assistant professor in 1960 and associate professor in 1964. He was an enthusiastic and hard-driving seagoing scientist dedicated to using ship-time to its fullest extent. In the same way he used his time ashore to the full, often hard at work until the small hours in his rambling and paper-filled house on the Hudson River and yet never too busy to devote time to his students. Neither did he stint himself in some of the pleasures of life. He was always good for a party, beaming, boyish in appearance and heavy in build. He had a strong and provocative personality, not mincing his words when he felt strongly about an issue, and it was tragic, but perhaps inevitable, that his early collaboration with the equally strong personality of Maurice Ewing should have changed into a bitter quarrel in which both parties as well as the science suffered.

Heezen's contributions to science have been recognised by the award of the Cullum Geographical Medal and the Francis Shepard Medal for excellence in marine geology, and in June this year by the award by the American Geophysical Union of the prestigious Walter H. Bucher Medal for his life's work of "original contributions to the basic knowledge of the earth's crust."

His loss will be keenly felt by his life-long collaborator, Marie Tharp, his many colleagues, friends and students, and by the whole marine geological community who owe so much to his energetic research, his insight into the deep oceans of the world and his inspiration of his students.

A. S. Laughton

## Howard Hinton

PROFESSOR Howard Everest Hinton FRS died on 2 August 1977, aged 64. He was one of the few remaining polymaths in the field of biology, having wide interests in many branches of the subject. However, he had a remarkable ability to become expert in any topic within a remarkably short time, so that few of his acquaintances, meeting him in connection with one facet of his interests, realised that this was not his major subject of study. Even his friends were often surprised by discovering his involvement in new fields of interest in science and the arts, as well as by his political naivety.

Hinton was born in Mexico, and his



reminiscences of his childhood suggested that his early education was far from conventional. It clearly did not allow him to achieve his academic potential, for he was not thought well grounded enough to enter the University of California at Berkeley, and had to content himself with admission to St José State College. However he so distinguished himself that he was soon able to transfer to the Berkeley campus. As a student he is said to have been aggressive and somewhat anti-social, with political attitudes and a concern for the underdog which, even at Berkeley, were forty years ahead of their time. Nevertheless he was clearly a brilliant student, and after taking his B.Sc. he moved to Cambridge, England to take his Ph.D. under the supervision of Dr A. D. Imms.

His main academic preoccupation at that time was beetle taxonomy, but his wider interests were already apparent. He went on scientific expeditions to his birthplace, Mexico, in 1933 and 1934, and in 1937 to Peru, Bolivia and Brazil. In 1939 he was appointed Assistant Keeper at the British Museum (Natural History) in London, first to work on what was to him the new subject of orthoptera (he soon made himself an expert) and then on the group which were still his main interest, beetles. He caused some concern to the authorities at the museum, for he was not prepared to adopt the gentlemanly working hours (10 to 5) then generally accepted. He slept—or worked—night after night in the museum during the blitz and often even when there were too few bombs falling to make this respectable. His scientific output was prodigious, including a 350 page monograph on the beetles affecting stored products in addition to a great many solid papers on insect taxonomy and phylogeny.

His work at that time included studies of larval lepidoptera, which led to a general interest in lepidopteran phylogeny. Some of his colleagues at the museum thought that he was devoting too much time to such studies and not to conventional taxonomy. Hinton was therefore glad to move, in 1949, to Bristol University as Reader in Zoology. This gave him greater freedom to choose his fields of study, a choice of which he made good use. He was elected FRS in 1961, to a personal Chair of Entomology in 1964, and became Professor of Zoology and head of the Department in 1970. At Bristol his best known research was his delicate work with the scanning electron microscope, with which he studied and elucidated the functional morphology of many forms of insects and their developmental processes.

Surprisingly for someone not brought up in the English countryside, Howard Hinton was an excellent field naturalist familiar with the flora and fauna of Britain. In fact he said that he derived his inspiration for his apparently esoteric laboratory work from his observations of living creatures in their natural environment. That his interests were not restricted to insects was further demonstrated by his production (with A. M. S. Dunn) of an attractive book on Mongooses.

As well as being an acute observer, a formidable synthesiser and a stimulating teacher, Hinton was an efficient and hardworking editor. He started and brought to success two major scientific periodicals, the *Journal of Insect Physiology* and the *Journal of Insect Biochemistry*. Most scientists would have felt that editing only one such publication was, in itself, a full-time job, yet Hinton managed both without any apparent reduction in his research or other activities. From 1969–70 he was also an effective President of the Royal Entomological Society, and in 1972 of the British Entomological and Natural History Society. Even towards the end of his life, when he knew that he was unlikely to survive until the date of his projected retirement in September 1978, he continued his activities with undiminished vigour and even completed his massive, three volume, monograph on insect eggs. His work in many fields will be appreciated for many years to come.

Kenneth Mellanby

## Leonard Eastham

PROFESSOR L. E. S. Eastham died on 19 July 1977 at the age of 84. Many former students remember him with gratitude and affection, for his understanding of their problems and his effective and unspectacular help. He came originally from Lancashire, and studied agriculture at the Harris Institute, Preston. This course was interrupted by the 1914–18 war, when he served in the Special Brigade of the Royal Engineers. After demobilisation he crossed the Pennines to Leeds, and studied zoology under Professor Walter Garstang. From 1921–27 he was lecturer in zoology at Birmingham University.

In 1927 he was appointed Lecturer in Advanced and Economic Entomology at Cambridge University. At that time Cambridge gave scant recognition to those coming from universities other than Oxford and Trinity College, Dublin (holders of provincial doctorates

were officially 'Mister') and Eastham's position was, at first, not a comfortable or an easy one. I remember him saying that some of his colleagues were so anxious to show their high social status that they forgot to behave like gentlemen. However, he was soon warmly accepted by his students, particularly those taking entomology in Part 2 of the tripos. He proved an inspiring teacher; his knowledge of agriculture, in a department where few had such practical experience, made the subject live, and a number of his students went on to important work in economic entomology in many lands.

From 1932 to 1958 Eastham was Professor of Zoology at Sheffield. Before the 1939–45 war the department was tiny, with only two other academic colleagues. Except for large classes of medical and dental students (1st M.B.) the number of undergraduates was not great, but there were separate classes for intermediate, general degree and special honours students, and a heavy teaching load which would overwhelm most present-day academics. Eastham bore his full share of the teaching, always maintaining his standards. He ran the department efficiently with next to no secretarial help. And he continued actively in research, producing a stream of scholarly and original papers.

After the war, the university, and the zoology department, grew rapidly to many times its previous size. Eastham was Dean of Science, and, from 1946–50, the first Pro-Vice-Chancellor. These and other outside duties did not prevent him from continuing his close and valued contacts with his junior colleagues and his present and past students.

From 1946 until his retirement Eastham played an important part in the creation and development of the new universities being established throughout the British Empire. In 1946 he was a member of a party under Sir William Hamilton Fyfe's chairmanship which visited West Africa and selected the site for the future university of Ibadan. He was a member of the Inter University Council for Higher Education in the Colonies (now for 'Overseas') and its executive, and he paid many visits to the embryonic universities and served on the Council at Ibadan. His visits were always appreciated; unlike many of the 'experts' who were similarly involved he always took the trouble to learn the facts, especially when political and other troubles arose, and his shrewd but sympathetic assessment helped and encouraged those trying to establish institutions of high academic standards under difficult conditions.

Kenneth Mellanby

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## No jobs left for the boys

A CRISIS is approaching in the employment of scientists in the academic and closely-allied worlds of the UK. Nothing explosive will herald its arrival; there will be no dramatic day on which we shall all wake up to find the newspapers full of it. Rather, over the next few years it is going to be more and more difficult for young people of an academic frame of mind to find a satisfying career. And no-one needs reminding that the influx of young staff is essential to the continued vitality of most academic departments.

The reasons for this crisis have often been discussed in these columns; they stem from three clearly defined factors. First, relatively few people are retiring from university posts at present, or, for that matter, from research-council laboratories or the scientific civil service. The enormous university expansion in the middle 1960s has left the system with large numbers of people now middle-aged: fewer than 1% of all university staff retire this year. The civil service has a somewhat more uniform age distribution, but even so less than 2% retire annually at present.

Second, for the past four years university finances have not allowed vice-chancellors the luxury of filling even such posts as have become vacant without asking the most searching questions about the prudence of taking on a new long-term commitment. The resultant partial freeze on hiring has nudged the student-staff ratio up to around 10:1, or from another point of view has called for an increase in the productivity of university staff.

Third, long term projections of student numbers unambiguously point to smaller universities in the 1990s. For the past ten years the decline in the numbers of those of university age (following the post-war baby boom) has been offset by a steady increase in the percentage of these who seek, and are qualified to receive, higher education. This percentage now seems to have peaked and to be in slight decline whilst the numbers in the relevant age group are rising to a new peak in 1981. For the past ten years then and the next five it seems as if substantial demographic swings will have been moderated by changes in the perceived desirability of going to university, resulting in a slow and controllable expansion in student numbers. But what happens beyond 1981 is anybody's guess. One thing is certain: that by the early 1990s numbers in the relevant age group will be more than a quarter down on those for the early 1980s. So the problem of adjusting university staffing to cope with this demographic change is a severe

one now, and will presumably become even more severe in the late 1980s as those educated at the time of the peak start to look for academic posts.

Thus, to get a job in 1977, you need to find someone who is retiring from a university that is not plagued by financial problems and is confident that in the long-term its student numbers will hold up. Needless to say you will not be the only applicant. And you will find the same competitors pursuing the same limited opportunities in research-council establishments. A supply of suitable people in excess of the demand for them must be one of the factors ensuring that low academic salaries hardly pose a headache to the government, despite mass lobbies and palpable inequities.

How do we get ourselves out of this bind, ensuring that bright minds are not wasted? Clearly some specific initiatives, such as support of more young scientists by the Royal Society, help to take a little of the strain. But it is unlikely that the problem will be seriously alleviated without some radical steps being taken—and in present circumstances these steps can hardly be vast expenditures on new laboratories. If they are we run the danger of acquiring yet another group which grows old together and doesn't start to open up further employment opportunities until the year 2010.

The unspectacular alternative (it can hardly be called a solution) is for very serious examination of the knotty problems of tenure and retirement. In the past ten years scientists, reflecting a national trend, have become ever more cautious of any sort of employment which left them without security above the age of 30, and have then felt safe until the age of 65. The civil service have, of course, led the way in providing a lifetime of stability. There is certainly a lot of good in buffering scientists from short-term change, but is the only alternative a thirty-five year guarantee of employment or, put another way, exclusion for thirty-five years of someone else from the same employment? Surely not when times are becoming progressively harder; some new form of contract of employment must be worked out which can respond to the aspirations of young scientists for employment without humiliating senior colleagues who have given a lifetime of service. Talks are already proceeding on these matters between various interested parties—those involved need every encouragement to come up with some flexible proposals as soon as possible. □

# Will the voice of science of the Third World be heard?

*Michael J. Moravcsik of the Institute of Theoretical Science, University of Oregon, appeals to third world scientists*

MORE than nine tenths of the scientific activity of the world takes place in countries comprising one quarter of the world's population, and most of the Third World at the present is virtually 'out in the cold'. The result is a strong inferiority complex, a necessity of subsisting on scientific and technological 'hand-outs' from the advanced countries, and a society which has not felt the impact of the dynamic world view that the practice of science can engender.

Some of the reasons for this state of affairs are internal to the developing countries themselves, some external. Much of science development must be done by indigenous manpower, with local resources. Other aspects of such a development process hinge on cooperation from the outside since science is a collective undertaking.

It is also a somewhat esoteric undertaking. Its aims, results, and needs do not automatically percolate into society which lacks the background and perspective to initiate interaction with science. Therefore scientists themselves must establish communication with people outside their ranks, and attract support for science making.

In this respect, again with some notable exceptions, the small scientific communities of the developing countries have been quite ineffectual in the past. There are two main groups who should have heard their voice: the policy makers in their own countries, and the world scientific community.

It is rare to encounter a developing country where decisions affecting the provisions for local science are significantly influenced by the active, working scientists of the country, instead of by civil service bureaucrats or by one-time scientists of advanced age who have long lost whatever contact and involvement they might once have had with actual science making. Such 'science policies' therefore are often restricted to the discussion of vague generalities, and in any case deal predominantly with technology and not science.

It is even rarer, however, to encounter a forceful and articulate voice from the Third World speaking out to the worldwide scientific community on behalf of the second class members of this community who happen to live in the developing countries. The large, international magazines used for communication among scientists seldom contain analyses and proposals for reform from representatives of the Third World and the large international conferences very rarely hear from such representatives about ways to make the practice of science more equitable throughout the world. The problem lies outside the consciousness of the overwhelming majority of working scientists.

## Opportunity: a UN Conference

The next two years appear to offer a favourable opportunity to effect a change in this state of affairs. Sometime in 1979 a huge United Nations World Conference on Science and Technology in Development will take place at which it should be possible for science in the Third World to speak out, should it decide to do so.

In particular, national position papers are being prepared, which discuss a country's needs and cooperative capabilities and put forward specific ideas and proposals with respect to any of the four main areas of the conference; science and technology for development, institutions, the United Nations system, and science and technology and the future.

It has been urged that the preparations of these country papers throughout the world be done in full consultation with all interested parties. Yet, it is quite possible that the

preparation of these most important documents in many countries is being done by a small group of people who are perhaps not even involved in scientific activities.

## An appeal

Thus the first appeal I would like to make is that scientists in developing countries make a most strenuous effort to partake in their own countries in the formulation of the country papers. There is no time to be wasted in doing so: the country papers are being written right now, and thus the input must be forthcoming promptly and forcefully. There are perhaps three main relevant areas.

- First, in the area of scientific education. Especially in the initial stages of the scientific development of a country, at least some of the indigenous scientific personnel must be educated abroad, and in this process cooperation from the scientifically advanced countries is an absolute necessity. The problems that arise are not only financial but also centre around the special needs such 'foreign' students have in the context of the education they receive abroad. Proposals have been formulated previously in a profusion of articles and meetings. Action is now needed.

- The second area of concern should be scientific communication. At the present all scientific communication channels are structured so that the scientifically rich get richer, and the scientifically poor poorer. Journal distribution, report and preprint circulation, personal visits, study leaves, conferences, and so on are all designed to yield scientific results tomorrow and hence neglect the yet evolving scientific institutions which promise to produce only the day after tomorrow. This subject has also been fully discussed in the past.

- Finally, and perhaps most importantly, one must create a worldwide atmosphere of opinion in which it is recognised that partaking in the practice of science is a legitimate, nay crucial, ingredient in the overall development process for all countries of the world. The condescending view that doing science is for the rich, and that the developing countries should rather strive to become better fed intellectual and technological slaves to the advanced countries must be changed. Development is a broad concept, and it includes equitability not only in GNP per capita, but also in the self-image of countries with respect to their overall contribution to and standing in humanity. Science development is a very significant element in this.

The second appeal I want to make to scientists in the Third World is to urge them towards articulation through an alternate channel. While much of the formal activity of the conference will be through the process of country papers, the atmosphere of the conference, the stand of individual delegates and speakers, the structuring of the discussion, and in fact even the details of the various country papers from the scientifically more advanced countries can all be significantly influenced by forceful communication through direct channels. Thus it seems that scientists from the Third World should 'campaign' by writing and speaking to fellow scientists the world over, to professional societies and journals, and to the conference organisers, the special office for the conference, headed by Joao F. de Costa, at the United Nations in New York. The more specific and programme-oriented statements and communication are, the more likely they will be assimilated into some action.

In summary, this appears to be the time for scientists of the Third World to rise and let their voice be heard. The psychological circumstances are favourable because of the huge build-up the conference is getting in political terms. The channels are also available. All we need now is action. □



# Combatting rickets

Researchers, nutritionists and policy-makers face a problem over rickets in Britain, as **Alastair Hay** explains

A QUICK survey of the British public would suggest that its nutritional problems are not due to a lack of food. Yet even in the midst of overconsumption a specific nutritional deficiency—that of vitamin D—has been defined in certain sections of the population. Rickets, known to generations of Europeans as the 'English disease' is now a problem in the children of Britain's Asian community and occurs sporadically in some of the infants of the country's poorest populations. It was first reported as being a serious problem in the Asian children of Glasgow almost sixteen years ago and has since been observed in one Asian community after another throughout the country.

The summer issue of *Science for People*—the publication of the British Society for Social Responsibility in Science (BSSRS)—asserts that the UK Department of Health and Social Security (DHSS) actually causes rickets. The journal is noted for a blunt approach to the issues it tackles, but this attack will not be viewed altogether unfavourably by many clinicians concerned with the health of Britain's Asian population and particularly with the problem of rickets. They point out that the problem is well known—perhaps too well known—and that there has been no shortage of surveys and quotable figures. They claim that most of the answers to this proven dietary deficiency are known, but that the DHSS has so far failed to adopt a positive approach.

Faced with such hostility the DHSS has been giving the matter serious attention. The department's Committee on Medical Aspects of Food Policy (COMA) is reviewing the whole question of bone disease contracted as a result of nutritional deficiency. The committee's deliberations have been somewhat protracted and are still by no means completed. But a report is expected that will produce some recommendations for action.

It is children who suffer from rickets; the same bone disorder in adults is termed osteomalacia. It results from an inadequate supply of vitamin D. The vitamin is required for the active absorption of calcium and phosphorus from the gut. When this mineral supply is reduced in vitamin D deficiency, the body, in order to maintain blood mineral levels, has recourse to its only other source of supply, the bones. Bone growth ceases, the skeletal structure become progressively weaker

and eventually deteriorates to produce 'bow-leg' or 'knock-knee' rickets.

Although biochemists are having a field day trying to elucidate vitamin D's exact mode of action, at the nutritional level the problem would seem to be simply that of ensuring an adequate supply. But the solution is not as easy as it seems, which could be one explanation why the DHSS is taking so long to formulate a policy. The question that needs answering concerns the most effective vehicle for administering the vitamin: should it be done through chapati flour, or milk, or vitamin D capsules, or tablets?

As a major constituent of the diet



of most Asian families, chapati flour would seem to be an ideal medium through which to add extra vitamin D to the diet. The technical problems of mixing the vitamin and of its preservation have been solved, and clinical trials using vitamin D-fortified chapati flour have shown its effectiveness as a method of administering the vitamin. A problem may arise with regard to the concentration gradient. It is children, not adults, who are most in need of extra vitamin D, and adults consume more flour per head. The answer would be to have two types of flour, one fortified and recommended for children, the other unfortified. But as a spokesman for a bread company says: "Someone has to pay for the process".

Milk poses different problems. There are no legal constraints to forbid fortifying chapati or brown flour with nutrients; this is not true of milk. Britain is bound by an EEC directive forbidding any alteration to the composition of milk. When the issue was discussed in Brussels, several European countries were in favour of legislating for a 3½% fat level. Britain fought this, primarily on financial grounds, and insisted on the right to have milk virtually unaltered from cow to con-

sumer (most milk would have to have fat removed to attain the 3½% figure). The British case was conceded, but at the time Asian rickets was not considered to be a problem; now it is. Before embarking on any milk fortification programme Britain would first have to return to the negotiating table, which could be embarrassing, but little choice may remain if the DHSS comes out in favour of using vitamin D-fortified milk. The arguments in favour of its use are strong. As children drink more milk than adults, it would reach the population most in need of treatment. Those in favour of milk point out that Canada fortifies milk, milk products and margarine with vitamin D; the United States does the same and much else besides.

As for the third suggestion that vitamin D capsules or tablets be dispensed either at clinics or in schools, staff in some schools are opposed on the grounds that the procedure of identifying children in need from the school register is too complicated.

As breast-fed babies in Britain rarely develop rickets any health education policy ought to discuss the merits of breast feeding as well. The reason for this immunity is not yet clear, but is probably related to the fact that the breast milk of vitamin D-replete mothers contains a fairly high concentration of the vitamin present as vitamin D sulphate. Some groups of immigrant women stop breast feeding when they arrive in the country and resort to doorstep milk.

## No significant impact

It is generally acknowledged that the health education programmes dealing with rickets have failed to make a significant impact in the Asian community. Some clinicians argue that these programmes have failed dismally in alerting local doctors and health workers to the seriousness of the problem. They add that there is great inertia at the level of the community physician, that many doctors are reluctant to become involved in preventative campaigns, and that many health workers have not been informed that the problem exists.

That rickets is present in the Asian community, and a serious problem, is not in doubt. Some may query the figures for the prevalence of the disease, and argue for more information. Others will say the evidence is now so overwhelming that action must be taken. The DHSS recognises that many doctors expect its COMA committee to make some positive recommendations stressing the need for a food fortification programme backed up by a good health education policy reaching all the Asian community. □



## More cash for British science

THE UK Advisory Board for the Research Councils (ABRC) is considering how to divide the second windfall to have landed in its lap this month amongst the five research councils. In reply to a question in the House of Commons late last week Shirley Williams, Secretary of State for Education and Science, announced an extra £4 million for the science budget which is to be spent on "new capital work" in 1978-79. It is in addition to the £4 million allocated to the research councils in the mini budget at the beginning of the month.

The new £4 million is to come out of the £400 million allocated to the construction industry in the mini budget. It is strictly for building and, because of its small size, is more likely to be used for improving or extending existing facilities than starting up new ones.

Professor Geoffrey Allen, Chairman of the Science Research Council, which is almost certain to get the lion's share, would like to see the money going towards finishing construction already begun and extending central user facilities. He feels that this might be an opportunity to complete the nuclear structure facility at the Daresbury Laboratory and extend facilities for the synchrotron source there. The laser facility and spallation neutron source at the Rutherford Laboratory might also come in for a share of the cash.

Another priority in the SRC is likely to be helping universities provide facilities for marine technology and polymer engineering. These are research areas considered to be of national importance and currently receiving special treatment from the SRC. The

only new project which might be considered is an electron beam lithography unit for microelectronics which could be set up in existing buildings at the Rutherford.

The cost of implementing these projects alone could come to £3 million. When the cost of the bids for support from all the research councils is added up, it will undoubtedly be much more than £4 million, so the ABRC is in for a difficult task. Nevertheless, it hopes to have made the necessary decisions by the end of next week or at the latest by its next formal meeting which is set for 9 December. A decision on the earlier £4 million, however, is unlikely to be made public before the end of the year although it has been made and is awaiting Mrs Williams' approval. It will probably simply be added on to the total science vote for 1978-79 which will be announced sometime in the new year.

**Judy Redfearn**

## Leading Soviet dissidents in London

DURING the last month, three leading figures from the Soviet dissidence and human rights movement have visited London. According to the most recent arrival, Dr Khronid Lyubarskii, an astrophysicist, their presence in the West reflects a determined effort on the part of the Soviet authorities to overthrow all opposition, neutralising the leaders by removing them from the scene. Neither Dr Lyubarskii nor Dr Valentin Turchin, the cyberneticist, who were expelled together on 14 October, had any real desire to leave the Soviet Union. They had applied for emigration only when the authorities had made it clear that they must choose between foreign exile or a protracted stay in a labour camp. The third visitor, Professor Mark Azbel, a theoretical physicist, had indeed had a genuine wish to leave the Soviet Union, having applied several years ago for a visa for Israel. After several years as a *refusnik*, Azbel finally received his visa last June.

It is no coincidence that all three are scientists. The dissident movement has a strong bias towards the scientific professions, since, in Dr Lyubarskii's words, "a scientist works in information—it is therefore natural that he begins to think about the society around him. It is the critical mind of scientists that leads them to think of human rights".

Their record of service to the human rights movement is impressive. As early as 1970 Turchin, together with Roy Medvedev, signed the second Sakharov letter. In 1972, Lyubarskii

was sentenced to five years in a strict-regime Labour camp for his human rights activities, and while there organised the observance of an annual Political Prisoners' Day (30 October), to be marked with protests and hunger strikes. For this further transgression, Lyubarskii was transferred to the Vladimir prison for the duration of his term. Both Turchin and Lyubarskii became members of the illicit Moscow group of Amnesty International, and were associated with the Helsinki monitoring group.

On the arrest of Aleksandr Ginsburg last April, Lyubarskii took over the administration of the Solzhenitsyn fund for aid to political prisoners. Unlike many Jewish activists, Dr Azbel feels it better to keep the Jewish emigration movement separate from the dissident movement for reform and human rights. This is not a distinction, however, which the Soviet authorities accept—application for emigration is an antisocial act, just as much as signing a petition of protest, and is punished in the same way by dismissal from one's job, with no possibility of continuing one's professional career.

Dr Lyubarskii gave an interesting estimate of the numbers of scientists penalised for their activities:

involuntary foreign exile	a few
arrests	tens
expelled from jobs	hundreds
routine restrictions after	thousands
signing protests etc (refusal of foreign passport, administrative restrictions).	

It should be remembered that punitive dismissal is far more serious a penalty than loss of job. In the Soviet Union no academic article can appear without a certificate from the academic institution where the author is employed. No 'freelance' submissions are permitted. "If Einstein or Faraday had lived in the Soviet Union, they could never have published their theories" stressed Azbel.

How well a scientist can survive professionally under such conditions seems to depend very largely on his particular field. In spite of several years without employment or professional facilities both Turchin and Azbel have found themselves able to continue their professional careers in the USA and Israel respectively. For Dr Lyubarskii, however, the outlook is bleak. His special field, the physics of planets, meteors and meteorites has moved forwards at such a pace that he feels doubtful whether he will ever catch up after almost six years with no access to journals.

Whatever their new professional commitments, all three are firmly committed to campaign for fellow-scientists still suffering constraint and imprisonment, and, indeed, they came to England for this purpose. They will campaign for physicist Yurii Orlov—now in prison; biologist Sergei Kovalev—in a labour camp; mathematician Viktor Brailovskii, Azbel's successor as leader of the Sunday seminar, who is threatened with a treason charge; and the young mathematician Anatolii Shcharanskii whose trial on treason charges now appears to be imminent.

**Vera Rich**



## Soviets honour teamwork and technology

It is sixty years after the revolution—but the 1977 Soviet State Prizes for Science and Technology show no sign of anniversary euphoria. The works cited seem genuinely to reflect the current state of progress, without any attempt to honour 'great names' on this special occasion. There is considerable emphasis on teamwork: the majority of citations involve long lists of project-directors, assistants, and technicians, so that the announcement of 38 awards with the names, academic or professional titles, and affiliations of the recipients fills a page of *Pravda*.

The citations span the usual wide range from mathematics to biology, with occasional incursions into fields not normally considered sciences in the West—Marxism and its ancillaries of history and economics. Current Soviet doctrine, as expounded at recent Party Congresses, demands that science should serve the national economy. Accordingly, several prizes go to research directly related to official priorities—a set of geological maps of Western Siberia, special equipment for land reclamation, fisheries equipment, and the use of discrete transformations in automatic control. The recent voyage of the icebreaker *Arktika* to the North Pole won a State Prize for her designers and engineers (though not for the scientific team aboard).

The medical sciences, which have recently received a new priority including the establishment of the title of merit "People's Doctor of the USSR", are well represented. Three science prizes go to long-term medical research: two—for haematology and prophylactic helminthology—mention "cycles of works" published over the last 15 years, while the third—one of the very few individual awards—cites a haematological monograph which also implies a long-term basis of research. Medicine also receives two technology prizes, where the citations seem rather more adventurous—new equipment for hyperbaric oxidation, and the "clinical development and implementation" of methods of limb transplantation.

In a year of setbacks for the space programme, it is not surprising to find space ignored in the technology section, although it does receive one science award—for research into the X-ray emission of the sun.

Nuclear research receives only one

award—for research into the hard X-ray fission of light nuclei. Commenting on this prize, the Chairman of the State Prize Committee, Minister of Higher and Secondary Specialised Education, Vyacheslav P. Elyutin, stated that "this work is of exceptional significance for the study of thermonuclear reactions and radioactive fallout". Nevertheless, in view of the repeated acclaim given in the Soviet media this last year to the fast-breeder power programme, it is a little surprising that there is no technology prize for nuclear engineering. The only other mention of nuclear physics is in the "textbook" section—for a two-volume university text on experimental nuclear physics.

This separate section for textbooks is a relatively recent innovation. In the Soviet Union, textbooks are selected by the educational authorities on a Union-wide basis, so that, on occasion, criticism of an unsatisfactory text will lead to a discussion in top-level party journals. The demand for scientific personnel is expanding with each Five Year Plan—the current Plan requires an "output" from the universities and institutes of higher education of 9.6 million new scientists, technologists and specialists in the period 1976 to 1980, so the provision of suitable textbooks is a matter of ever-increasing importance. This year two other university textbooks, on pedology and invertebrate palaeontology, are also honoured, as is a secondary school text on economic geography.

Vera Rich

## Recombinant DNA

THE final Environmental Impact Statement (EIS) on the NIH guidelines for research involving recombinant DNA molecules was published last week, more than 16 months after the release of the guidelines themselves. The formulation of the final EIS has been fraught with delays and uncertainties. An initial draft which came in for some heavy criticism was published over a year ago along with an invitation for informed comment. Drafting an acceptable final version has taken much longer than expected, and the publication schedule has slipped several times. This delay may have legal consequences. Friends of the Earth has filed a suit against the NIH and NSE in an attempt to halt funding of recombinant DNA research, charging breach of the National Environment Protection Act on the grounds that the EIS was not published simultaneously with the guidelines. The NIH counter this by pointing out that delaying publication of the guidelines would have led to greater delays in applying adequate safeguards to the research.

As for the EIS itself, there are few surprises. The document and its extensive appendices go over all the familiar arguments concerning the potential and postulated environmental impacts of recombinant DNA research and comes to the unsurprising conclusion that "the level of risk that will result . . . is acceptably small." This conclusion and the arguments used to support it will undoubtedly supply ammunition to both sides of the continuing debate.

Sandy Grimwade



Above: the Meridian Room at the Dunsink Observatory of the Dublin Institute for Advanced Studies, where a major fire early last October severely damaged the library and laboratories. Loss of instruments has meant loss of valuable work. But Professor P. A. Wayman, Director of the Observatory, is hopeful that the observatory will be restored. He sees as first priority getting the smoke damaged computer back into action. Restoration of the eighteenth century building which housed the library is likely, though not definite yet.

The Observatory was founded in 1783 and became part of Dublin University. In 1947 it was taken over by the Dublin Institute. It has no telescopes of its own.

Chris Sherwell, News Editor of Nature for the past two years, has taken up a post on the foreign desk at the Financial Times. Robert Walgate, until recently with New Scientist, takes Mr Sherwell's place. And David Dickson, recently with the Times Higher Educational Supplement, becomes our Washington News Editor at the beginning of 1978.



## Austrian science minister attacks science journalists in fraud case

THE Austrian Minister for science and research, Dr Hertha Firnberg, released a 32-page document last Wednesday (16 November) attacking the science journalists of Austrian and other newspapers for poor judgment, and for unwittingly assisting in the execution of a crime. The attack, whether justified or not, threatens to disrupt the relationship—already fragile—between Austrian journalists, the ministry and the scientific community.

The government statement singled out two journalists for particularly harsh treatment: Dr Eleonore Thun-Hohenstein of the conservative daily *Wochenpresse* and Paul Uccisic of the middle-of-the-road *Kurier*. Their error? Publicising the work of a little-known freelance microbiologist, Herbert Schaden, and his assistant Hertha Celta. Schaden and Celta claimed that they had developed, or would soon develop, a strain of microbe which would digest plastic. No such strain emerged after four years' work.

Schaden was detained in Austria on 11 January, and has been in jail since, awaiting trial on charges of fraudulently obtaining funds for research (amounting to some £½ million) from a Dutch packaging firm and other sources. Celta was imprisoned with him but released on bail after two months—to feed Schaden's microbes. The microbes and Celta languish yet in Schaden's private laboratory at 99 Panzingerstrasse, a fashionable turn-of-the-century Viennese villa.

Dr Firnberg's document implies that Schaden is a crook—albeit a very clever one—and attacks the journalists for helping him along the path to fraud. Some 750 articles describing Schaden's work appeared in the press between 1 February 1973 and 5 June 1974, the document states, mostly in Austria, but also in Holland and in Italy.

Schaden first came to the journalists' attention on 1 February 1973, when he sent a thick bunch of documents to the Austrian *Bundersministerium für Wissenschaft und Forschung*, with copies to the Austrian newspapers. The documents gave credentials, references, and evidence that Schaden and Celta had found the plastic-digesting bug. Years before, a stack of plastic containers had been found riddled with microscopic holes; Schaden had deduced the presence of bacterial action and set about isolating the active agents.

On 21 February 1973 Thun-Hohenstein's first article, a positive one, appeared in *Wochenpresse*. A Dutch journalist noticed the story and

relayed it to her paper, the *Haarlems Dagblad*, a small but important provincial Dutch paper.

Oscar van Leer, a Dutch packaging magnate, came to hear of the story and was interested. Uccisic published his version of Schaden's work, also positive, in *Kurier* on 4 March. By 30 May Thun-Hohenstein had heard both that the Austrian ministry had dismissed Schaden ambiguously with a "don't ring us, we'll ring you", and that van Leer's firm (Royal Packaging Industries van Leer B. V.) was investing in Schaden's bugs. van Leer had sent a team of plastics experts—but not microbiologists—to interview Schaden and concluded he was genuine. Thun published a sarcastic article ridiculing the ministry's delay and poor judgment. On 9 June Uccisic, not a man to mince words, weighed in with his own criticism. He printed a story related by Thun: that the chief civil servant responsible for advising on the allocation of research funds in Austria—Dr Wilhelm Grimburg—had threatened Thun with a court action for saying the ministry's rejection of Schaden took five lines rather than the five and a half. Uccisic made great play of that. Later a letter from Grimburg was printed in *Kurier* rebutting Uccisic's accusations; but Uccisic tacked a stinging comment on the end.

Grimburg's relationship with Thun-Hohenstein and Uccisic was therefore very sour; the more so because the ministry's popularity with scientists was at a low ebb. Austria was—and is still—enjoying its first period of socialist government for a long while, and the ministry had embarked on a "demo-

cratisation" of the universities (for which it is responsible), introducing students and junior professors on to decision-making bodies, and dismantling the previous patriarchal and authoritarian structures. Criticisms of competence were particularly unwelcome at that time.

But now has come the retribution. van Leer invested a large sum in Schaden, only to find his work and his claim valueless. van Leer's lawyers believe they have discovered that some 40 pages of Schaden's original documentation are careful re-writes of earlier German papers, changing names and places. So the ministry appears to be vindicated (though Schaden and Celta's case has yet to come to court, and some feel that due process of law has been disturbed by the ministry's attack). Dr Firnberg, the minister, threatens wide distribution of the report, to all politicians and universities in Austria, an act which will undoubtedly severely weaken the position of the science journalists within their own newspapers and in Austria itself.

What of the journalists? Dr Thun-Hohenstein dismisses the Firnberg attack and indeed still believes in Schaden; Herr Uccisic thinks Schaden "not the person we took him for". He feels however that "neither I nor Dr Thun-Hohenstein nor anyone has any proof" that Schaden is wrong. And many journalists are saying that as it has taken experts since 11 January this year to develop a watertight case against Schaden—and may take longer—how can they have been expected to judge? The development was potentially of great public interest so had the public not the right to know?

Uccisic also takes a more philosophical view. "Error is always possible" he says; "even politicians and scientists can make errors". Uccisic believes scientific officialdom to be too hidebound—which it sometimes is—and he takes an interest in fringe science. But the belief can lead one into dangerous territory. Uccisic recognises the danger but adds "We should be open-minded and ready for new developments. In this special case no law of science forbids the development of organisms that might digest plastic. And Schaden was employing a recognised technique: to isolate a useful strain and strengthen it by multiplication and selection".

The final criticism must be laid at the door of the ministry itself. The ministry accuses the journalists of superficiality and lack of thoroughness. But the document they have produced in their haste to attack the journalists suffers from the same fault. It is too polemical; and its puts only one side of a difficult case.

Robert Walgate



Herbert Schaden and Hertha Celta, accused of obtaining £½ million by false pretences



## EEC taps a megawatt of Italian sunshine

THE EEC Commission and a consortium of European companies signed a construction contract in Brussels last week for a £3 million electricity generating plant operated by the sun. The plant will probably be built in the south of Italy, and should be producing a megawatt of electricity by 1981—enough power for between 100 and 200 households.

Two hundred and fifty mirrors, covering a hectare of ground, will be used to reflect and concentrate the sun's heat on to a boiler, on top of a 50-metre-high tower, to produce steam to drive conventional turbines. The heat receiver will be about four metres square. Each square metre of mirror will generate 1 kW. The plant will have a thermal power of about 5 MW indicating a thermal efficiency of 20%. It will operate with steam at 510 °C and

64 atmospheres. The mirrors will be computer controlled to follow the sun and the whole structure will be solid enough to withstand winds of up to 130 km h<sup>-1</sup>.

This will not be the first such solar electricity generating plant. France, which is taking part in the project, already has its own 2 MW plant near Marseilles and Macdonnel Douglas in the USA is planning to build a 10 MW plant in California at an estimated cost of \$31 million. But because of spending cuts that is likely to be delayed.

All these plants, however, will be expensive, experimental projects, operating on an uneconomically small scale. The maximum possible output for a solar plant is thought to be about 100 MW. Beyond that it becomes physically impossible to get more mirrors close enough to the boilers to

have any effect. Major modern electricity generating stations can produce 1,000 MW, and Mr Albert Strub, head of the EEC Commission's non-nuclear energy department, estimates that "if 5% of Europe's energy comes from the sun by the year 2000, we will be doing well".

The aim of the Italian plant is to teach the companies concerned the technical problems involved. The firms concerned are: the Italian national electricity company, ENEL; Ansaldo of Italy; Cethel (grouping the French companies Renault, St Gobain and Heurtey); and Messerschmitt-Bolkow-Blohm of West Germany. General Technology Systems of London will advise the Commission on the management of the project. Half the cost will be met from the Commission's research budget, the remainder coming from the participating countries.

Brian Donaghy

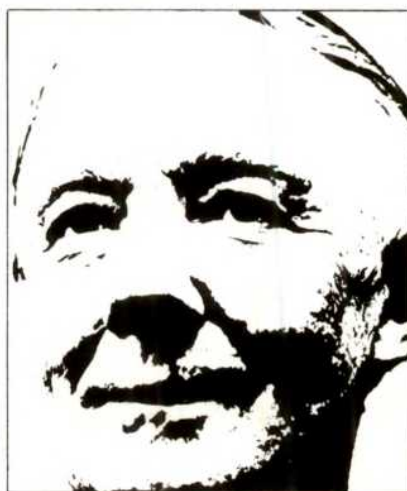
THE Royal Society of Tropical Medicine and Hygiene is holding a symposium this year, from 23 to 25 November, to celebrate the first centenary of medical entomology. It is just one hundred years since Patrick Manson showed that *Culex fatigans*, the common house mosquito of the tropics, was the intermediate host of a nematode worm which caused the disease filariasis in man. That was the first time an insect had been so involved, and although other scientists were engaged in parallel investigations, and there have been unpleasant confrontations between those supporting other claimants to priority, I have no doubt that Manson fully deserves this accolade.

The novelty of his discovery is manifest when we examine the records of his time. The more traditional members of the medical profession called him a lunatic, and intended to ridicule him when they gave him the nickname 'Mosquito Manson'. However, he inspired a few of his colleagues, particularly Ronald Ross. To the general public Ross, the discoverer of the transmission of malaria by the mosquito, was the more popular figure, but Ross himself always acknowledged his debt to Manson.

I do not think that it is any exaggeration to say that Manson's work has had a more profound effect on the human race than any other discovery which can be attributed to an identifiable individual. We cannot name the benefactors who discovered the wheel, the use of fire and the possibility of growing arable crops: their contributions may have been more revolutionary to man's way of

life, but the results are of the same order of magnitude. But for the work of Manson and those he inspired, the populations of many tropical countries might today be less than half their present level.

### Mosquito Manson



KENNETH MELLANBY

A hundred years ago the majority of the world's population lived in areas where diseases which we now know to be transmitted by insects (and in no other way) dominated the lives, and the deaths, of the inhabitants, yet no one had the faintest idea how the diseases might be controlled. Even today malaria, typhus, sleeping sickness, filariasis, dengue and many more diseases are still important, claiming many victims and affecting all types of development, but we know how they could be reduced or even

eradicated. Though patchy, some degree of control has been widely implemented. The resulting rise in population may not be an unmixed blessing in a world of shrinking resources, but to many individuals, particularly in tropical countries, the improvement has been dramatic.

Although a hundred years ago no one appeared to believe that insects could carry diseases, it is tempting to search for earlier suggestions of such a mechanism. It is true that authoritative books on the English fens produced in the nineteenth century which devote much space to "the ague", which was generally malaria, give no inkling of any idea other than that the disease was caused by the "bad air" from the marshes. They discuss the importance or otherwise of water pollution by organic matter, which can now be interpreted as affecting the survival of mosquito larvae, but there is no mention of mosquito bites as a danger to health. However, I recently found a contemporary account giving an inventory of the contents of the Cistercian Abbey at Sawtry, only a couple of miles from where I write in Huntingdonshire, at the time of the dissolution of the monasteries in the sixteenth century. The Abbot's chamber had a feather bed and white curtains, but the guest room, reserved for important visitors, was hung with tapestry and a bedstead with "nets for knats". This may have been to protect the guests from infection with malaria, but I fear that it was just for his comfort and to keep out the insects which can still be troublesome on warm summer nights in fenland. But it may have kept him healthy all the same.



# correspondence

## Farm energy

SIR,—I am not sure what your correspondent Michael Knee is trying to say in his letter (13 October, page 556). He accuses me of "blind prejudice" for stating a few simple facts about the use of energy by farmers. If he reads my contributions in the issues of 25 August (Frugal farming) and of 8 September (Food for energy), he will find that he has misinterpreted my views.

I would be the last to deny that an enormous amount of energy is wasted by some modern farmers, but this is almost entirely because of the inefficiency of intensively-kept livestock, which waste over 90% of their food, and which is the main reason why Britain is not already largely self-sufficient. This is mentioned in my contribution of 25 August, and explained in detail in my book *Can Britain Feed Itself*. But this is irrelevant to the present argument.

My contention is that arable farming is efficient. It has been shown by everyone who has investigated the situation, including Gerald Leach in his *Energy and food production* (mentioned by Michael Knee) that arable farming, although using much energy to propel tractors and manufacture fertilisers, produces crops with at least three times the energy value of that used in growing them. Recent developments, such as direct drilling, will reduce energy expenditure without reducing the yields, and so further improve the equation. In fact intensive arable farming is probably the most efficient way we can trap solar energy in an easily useable form.

Like Michael Knee, I would like to see more people working on our farms, but men, like horses, would probably use more energy in a twelve-month than the present tractors and combine harvesters, if we were to grow and harvest comparable crops.

Yours faithfully,

KENNETH MELLANBY

Huntingdon, UK

## Desert rainfall

SIR,—The statement by Glantz and Katz (19 May, page 192) with regard to measuring rainfall at two locations, Gao and Niamey, in the Sahel "that at least is some sense, the mean is too large and not at all indicative of how much rain commonly falls" is overly

strong. In no sense is the mean too large; the mean is simply the mean—a well defined and much used statistic. Furthermore it is quite indicative of how much rain commonly falls in most regions—even Gao and Niamey. Glantz and Katz also note that positive "skewness is characteristic of the rainfall distribution not only in the Sahel but, more generally, in arid and semi-arid regions. In particular the degree of skewness is greater the drier the climate". It has been our experience, however, that positive skewness is a characteristic of rainfall distribution for almost all localities and that, at most, there seems to be only a slight tendency for the degree of skewness to increase as the average amount of precipitation decreases.

We selected a random sample of fifteen reporting localities from the original *Climatic Summary of the United States* which contained data from the establishment of reporting stations up to 1930. The only restrictive requirement imposed in the selection process was that there had to be at least twenty consecutive years of records for a given locality. We used the mean, standard deviation, and coefficient of skewness for the distribution of precipitation at each location to examine the hypothesis that the more arid the climate the more skewed the distribution rainfall. If the hypothesis is valid, there should be a strong negative correlation between mean rainfall and the skewness coefficient. We found that there did seem to be more skewness for regions with little rainfall in the month of July than for a region of average rainfall, but positive skewness also seemed to be greater for regions with greater than normal rainfall. The correlation coefficient of  $r = -.08$  tends to confirm the lack of a simple systematic negative relationship between mean rainfall and skewness.

It also should be noted that positive skewness seems to be the normal condition for the distribution of rainfall for almost all localities and not just for the localities in arid regions. The meaning of this should be evident. If it is not true that the mean alone is suitable for describing the distribution of rainfall in arid regions, then neither is it alone a suitable measurement for describing the distribution in any region. This, however, is not a surprising result; few distributions can be de-

scribed by one parameter. As Glantz and Katz state, "no single number can adequately describe the climate regime of an arid or semiarid region". We would add that the statement holds for all regions and not merely for arid or semiarid regions.

We also would like to add a note of disagreement with the statement that, "recent weather tends to influence perceptions more heavily than earlier weather and wet spells more heavily than dry ones". While the statement about recent weather versus earlier weather seems totally acceptable, the latter part of the statement would seem to need at least some documentation. What evidence exists to substantiate this statement? If nothing else, it does seem evident that the drought of the past few years in the Sahel will influence perceptions and behaviour quite as much as the earlier wet spells.

Yours faithfully,

J. LARRY DEATON

United States Department of  
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Washington DC

## Using varied talents

SIR,—I was unpleasantly surprised at your negative editorial comment on Sir Andrew Huxley's Presidential Address (8 September, page 95). Accepting the idea that inherited differences exist does not automatically condemn the apparently less-endowed people to a subhuman status. Those with no apparent talent are not necessarily inferior; they simply can't find their true calling in our culture. A century ago born atomic physicists and computer programmers may have spent their lives on the skid row as impractical silly dreamers; today they are productive, well-regarded members of our society.

As civilisation advances, more and more people will find the calling that uses their innate talents; fewer and fewer will spend their lives as frustrated misfits. All we have to do is to maintain progress in our civilisation, so more and more varied talents can be used. However, to maintain this progress we must have the courage to face the apparently impalatable as well as the palatable results.

Yours faithfully,

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USA

# news and views

## DNA insertions and gene structure

from Bob Williamson

ONCE again we are surprised, this time by the structure of genes coding for proteins in higher animals. The primary sequence of the protein is determined by messenger RNA (mRNA); this in turn is synthesised on one of the strands of genomic DNA, probably first as a precursor of high molecular weight (HnRNA). Several animal cell mRNAs have been sequenced in whole or part, revealing no peculiarities so far: the coding sequence is preceded by a capped, non-translated region, starts with an initiator triplet, and is read in phase to the 3'-terminator triplet, which is followed by a non-translated 3' region and last, a polyadenylic acid sequence. The sequence of the coding region of the mRNA exactly specifies the amino acid sequence of the protein when read in register.

It was assumed that the mRNA was copied directly from a gene sequence, and data from prokaryotes certainly supported this view—a frame-shift mutation in which a base was added or deleted caused the predicted change in protein sequence, and even, in an operon, in proteins coded adjacent to the 3' side of the genome. In animal cells, however, this now turns out not to be the case, at least for ovalbumin and  $\beta$ -globin genes.

These genes are particularly suitable for study as they are present only in single copies, and tissues which make each protein in large amounts can be compared with those that make little or none. The specific mRNAs are available, and the construction of recombinant DNA plasmids has removed the problems of purity and amount that particularly apply to single-copy genes because they are present in the sequences in nuclear ('genomic') DNA only as one part in several million.

No sooner was an appropriate recombinant clone of DNA available (courtesy of T. Maniatis) than Flavell and Jeffreys started the analysis of the genes for rabbit  $\beta$  globin. They 'mapped' the gene—that is they found the size of the DNA fragments containing the gene when the DNA was digested with various restriction enzymes by techniques developed by Southern (*J. molec. Biol.* **98**, 503; 1975). They looked for the nearest repeated DNA sequences, the nearest short poly(dA) runs, and so on. It all made sense for a while—the gene for  $\beta$  globin (there are actually two related genes, which seem to correspond to the  $\beta$ - and  $\delta$ -globin genes in humans) at first behaved correctly, and a picture of the gene itself, the surrounding bits of DNA and the minimum distances to other genes became partially clear (Jeffreys & Flavell, *Cell*, **12**, 429–441; 1977).

Then more enzymes were used. When a variety of enzymes is used, in a way analogous to that in which proteases are used to determine protein sequences, they have to 'add up'—if sequence AC is cut at point B, then AB plus BC has to equal AC. This is so 'self-evident' that Jeffreys and Flavell deserve the greatest praise for not dismissing their data out of hand when they showed that 2+2 does not always equal our preconception of 4.

In the  $\beta$ -globin mRNA, the sequence of bases starts at one end with the triplet specifying the N-terminal amino acid of the protein and moves steadily and without interruption to the other end, where the protein terminates. But in the gene the sequence of bases coding for  $\beta$  globin is not continuous. In particular one fragment of cloned complementary DNA (obtained by reverse transcription of the mRNA) which is 333 bases long, derived entirely from the coding sequence and bounded by two *Hae*III restriction sites, hybridises to a *Hae*III restriction fragment of genomic DNA that is more than twice

as big. Clearly the restriction fragment contains an additional insert (some 700 base pairs long). The only conclusion must be that there is a bit of DNA, as big as the structural gene for  $\beta$  globin itself, in the middle of the gene, which does not appear in the final mRNA (Jeffreys & Flavell *Cell*, in the press).

At the same time as Flavell and Jeffreys were obtaining this startling result, Leder *et al.* were recording a related and equally inexplicable finding (*Cold Spring Harb. Symp. quant. Biol.*, in the press). Leder's group had first to prepare a safe vector to clone mammalian genes, and chose bacteriophage  $\lambda$ . They introduced a number of mutations and deletions so that only recombinants could be 'packaged' and so reproduce, and also several mutations which rendered the phage non-viable outside controlled laboratory conditions. They then purified the gene for mouse  $\beta$  globin several hundred times using a combination of reverse phase chromatography (which separates on base composition and secondary structure) and Agarose gel electrophoresis, and obtained two fragments approximately 15,000 and 7,000 base pairs (15 kb and 7 kb) long. Each of these, while much purified over the original DNA, still only contain one globin gene-containing sequence in every thousand or so total sequences.

The mixture was then recombined with the disabled  $\lambda$  vector. Recombinants containing the mouse  $\beta$ -globin gene were selected. The length of the cloned mouse DNA was ten times the size of the coding sequence, some 7 kb long, and contained the entire  $\beta$ -globin gene. However, once again, it was not all in a single piece, for when it was hybridised to mouse globin mRNA and examined with the electron microscope, a remarkable extra loop in the middle of the DNA was found. Restriction enzyme analysis confirmed the existence of the insert. This insert is 450 base pairs long—it is not yet clear whether it is in the same position as

for the rabbit insert described by Flavell and Jeffreys, nor whether its sequence is conserved.

The chicken ovalbumin gene has been studied almost as extensively as the globin gene, largely because it is hoped to learn from it more about gene action in response to hormone stimulation. Because it is present only in a single copy, fragments of genomic DNA prepared using a specific restriction endonuclease which does not break the gene should once again give a single DNA fragment containing the gene—if the sequence is present as a single unit in the DNA as in the mRNA. However, two groups have just shown that as for globin, several fragments rather than one are often found (Breathnach, Mandel & Chambon, this issue of *Nature*, page 314; Doel, Houghton, Cook & Carey, *Nucleic Acids Res.* **4**, 3701–3713; 1977).

Chambon and his colleagues first prepared a recombinant plasmid between complementary DNA (cDNA) prepared from chick oviduct ovalbumin mRNA and pCRI. This enabled the Strasbourg group to determine which restriction endonucleases did not cleave the coding regions of the gene.

A slightly different approach was used by Carey's group at Searle. They first prepared very pure mRNA and isolated full length cDNA, and then synthesised a second complementary strand with reverse transcriptase. Full length double strand cDNA was isolated by Agarose gel electrophoresis. This too could be checked for restriction enzyme sites in the coding gene region. (Carey *et al.* also checked their results against the plasmid prepared by the Strasbourg group, a good example of international co-operation in the use of recombinant DNA.)

Neither of the restriction enzymes *EcoRI* nor *HindIII* cleaved the structural gene sequence in Chambon's plasmid or Carey's double-stranded cDNA. These then were the enzymes used to fragment genomic DNA from chick tissues.

Both groups, using their different techniques, found three DNA sequences which hybridised to either cDNA or nick-translated plasmid DNA after restriction of genomic DNA with *HindIII*. Using *EcoRI*, Chambon *et al.* found convincing hybridisation in four positions. Thus instead of a single band expected, several gene-containing regions separated by long inserts, or spacers, are found. Moreover, this is true for all tissues studied, whether making ovalbumin or not.

In the case of mouse and rabbit  $\beta$  globin, the entire coding gene was isolated, with the insert in the middle. The structural gene regions were therefore at least closely linked, if separated by the unexplained sequence. In the case of ovalbumin, however, no frag-

ment containing both coding sequences has been found and one of the inserts is at least 7,000 base pairs long. It is even theoretically possible that different regions of the ovalbumin structural gene are on different chromosomes, and not linked at all.

How can this remarkable result help in the understanding of transcription? Very little, until several questions have been answered. As Chambon *et al.* point out today, there are three apparent possibilities. The 'primary' transcript (the HnRNA containing the mRNA sequences) may include the inserts and all the mRNA coding regions, with the inserts being specifically nicked out and the mRNA ligated to its final form in processing. This is quite possible because, as has been stated here (*News and Views* **269**, 648; 1977), the size of the globin 'precursor' most easily identified for  $\beta$  mRNA is 15S, just the size expected if the insert is still present flanked by 3'- and 5'-coding regions. However, such a correlation is at best indicative. The concept of a transcribed insert is also, however, supported by the puzzling fact that both the poly (A) sequence at the 3' terminus of the mRNA and the methylated 'cap' at the 5' end seem to be conserved from the HnRNA to the final polysomal mRNA. The explanation of this is clear if the processing occurs not from one end or the other, but out of the middle.

If the inserts are transcribed, there must be a very accurate enzymatic mechanism for cutting them out. Such excision enzymes are known (Robertson & Dickson, *Brookhaven Symp. Biol.* **26**, 240–266; 1975), but have never been demonstrated in animal cells, although ribosomal RNA processing certainly involves similar accurate cleavages. The inserts might be expected to contain extensive self-complementary double-strand regions, such as palindromes, but they do not self-reassociate (at least for rabbit  $\beta$ -globin genes), indicating that if such sequences are present, they are quite short. Also, the inserts may be repeated a few times throughout the genome (Flavell and Jeffreys feel 10–15 times is the highest reiteration frequency their data might support), but are certainly not common to many structural genes. Perhaps the excision specificity is mediated by nuclear protein which, in interacting with HnRNA, would both force certain RNA conformations and also act as additional markers for specific enzymes—or even have enzymatic activity themselves.

The second possibility is that looping out occurs at the DNA level, and the inserts are never transcribed at all. The main attraction of this idea is that, while we know little about DNA structure in active chromatin, most pos-

tulates are still possible. (It also would help to explain the difficulty in obtaining convincing and reproducible transcription from chromatin because additional factors would be required to control DNA conformation for active genes.)

Finally, it is possible that the portions of the mRNA are synthesised separately and then ligated together during processing. In this case the 'inserts' might or might not be transcribed, but the various bits of the coding sequence would not be co-transcribed in a single long piece of RNA. Interestingly, the gene for which the best evidence exists for the absence of HnRNA is precisely that reported here—McKnight and Schimke (*Proc. natn. Acad. Sci. U.S.A.* **71**, 4327–4331; 1974) demonstrated that it is very difficult to show an ovalbumin HnRNA, using techniques which seem to work for globin.

No doubt we shall soon know whether the 'insert' is transcribed, and perhaps have definitive data on its multiplicity in the genome and whether it includes short palindromic signals which might indicate looping-out sites. Such experiments will be most interesting for globin, where chromatographic techniques allow the isolation of precursor RNAs (*News and Views* **269**, 9; 1977).

It is very interesting to compare these data for globin and ovalbumin genes with that reported last month in these columns (*News and Views* **269**, 648; 1977) for mouse immunoglobulin genes. In the case of Ig, there are also inserts, in DNA from antibody-synthesising tissues and from other tissues, but the inserts appear different, and at least some somatic gene reassortment takes place. For globin and ovalbumin there is no apparent difference between tissues making the mRNA and those completely inactive for the protein in question. The inserts may allow novel somatic recombination events to occur, but do not make them obligatory. It is also unclear whether these sequences are related to the leader sequences which are transcribed from remote regions of the genome in adenovirus and SV40 (*News and Views* **268**, 101; 1977).

Let us briefly consider some of the wilder possible implications of this finding. If transcripts are ligated together during processing, there is no need that they be arranged in the 'correct' order—from 5' to 3'—in the genome. In fact, genes could exist with a single 5' end and several 3' ends, which are used interchangeably or in sequence during development. Maybe the  $\beta$ - and  $\delta$ -globin genes or the two  $\alpha$ -globin genes share some parts in common, only the 'different' bits being ligated in the right proportions. If transcribed, the inserts must be (how

easily we fall into such formulations, in spite of these findings) nicked out intact; the more reason to think they could have other regulatory functions. However, if not transcribed, surely as indicated above, the inserts are now the best candidates for marker sites for binding gene-specific non-histone proteins, and thus determining chromatin conformation during the control of transcription.

Those of us who are interested in safety with recombinant DNA sequences are left with the following thought. Until now, it has been assumed that cDNA derived from an mRNA is less likely to be expressed

(and therefore, in some contexts, might be regarded as 'safer') than a total gene sequence, including the flanking regions. Now with the discovery of gene inserts it is quite likely that the controls involved in transcribing and/or processing several different bits of DNA into highly complex precursors of RNA, followed by excision and ligation steps, will be beyond the capabilities of the bacterial hosts used for recombinant DNA vectors. Hence the genomic DNA recombinants may prove to be just as safe as cDNA recombinants unless much work goes into inserting processing systems as well. □

which is much the strongest emission line in the solar spectrum. The coincidence between the Lyman- $\alpha$  frequency and that of the nearby line in the  $H_2$  spectrum is not exact but the Lyman- $\alpha$  line is so broad that there is overlap.

It is natural to ask whether there are other molecular fluorescence spectra waiting to be found in astrophysics. A general argument suggests the chance is high—molecular energies and transitions are numerous because of the many rotational and vibrational states, so that the chance of a coincidence with a transition of an atom or another molecule is much higher than for atomic spectra. It is, for example, not out of the question that a specific fluorescence process might be responsible for inverting populations of levels in the ground state of a molecule from which microwave stimulated emission occurs (Cook *Nature* **210**, 611; 1966); indeed the process suggested by Litvak (*Astrophys. J.* **156**, 471; 1969) for infrared pumping of the hydroxyl maser is of this type, although it relies on the hydroxyl itself to filter continuous radiation and produce a distribution of intensity that gives rise to selective fluorescence. Furthermore, as Jordan and others themselves mention the selective absorption of Lyman- $\alpha$  by interstellar molecules may be important in the formation of interstellar spectra of molecules (Osterbrock *Astrophys. J.* **136**, 359; 1962; Black & Delgarno, *Bull. Am. Astron. Soc.* **6**, 444; 1974).

The other question which is raised by the analysis of Jordan and others is the part played by molecules in stellar atmospheres. If molecules are at all abundant they will affect the absorption of radiation in an atmosphere in two ways. In the first place, there will be continuous absorption leading to dissociation and the frequencies for dissociation are in general much less than those for ionisation of atoms or molecules. Second, molecular lines often lie so close together because of the many rotational and vibrational states that exist, that in some parts of the spectra absorption in discrete lines will come close to continuous absorption. Molecules will of course be dissociated if the temperature of the atmosphere is high but many molecules can exist at temperatures up to 3,000 K. It has indeed been suggested that hydroxyl may contribute to the opacity of the solar atmosphere, and it is almost certain that  $H_2$  is present in cool stars where molecules such as  $C_2$  and CN are already observed.

The study of Jordan and others and the ideas which stem from it call attention to the importance of molecular spectra in the neighbourhood of Lyman- $\alpha$ . Because the energies lie above the dissociation energy of most

## Molecular hydrogen in the Sun

from A. H. Cook

A RELATIVELY large proportion of matter in galaxies lies in the space between stars and until fairly recently appeared to be in the form of free atoms and solid grains. Atoms were detected in two ways—by the microwave radiation from the hyperfine transition in the ground state of hydrogen at 1,421 MHz and by absorption of starlight by atoms showing up the spectra of the stars in the visible region. Solid particles scatter, polarise and absorb visible light from stars. There was also evidence for the existence of a few molecules, CH and CN in particular, absorption lines of which lay in the visible. It was also well known that some molecules, such as  $C_2$ , TiO, and CN occurred in cool stars. By and large however, limitations on the sensitivity of radio measurements and the restriction of optical spectroscopy to the visible meant that direct evidence of the existence of molecules could not be obtained. At the same time, dynamical considerations indicated that there was more mass in the Galaxy than could be accounted for by the observed hydrogen and other atoms.

So far as interstellar material goes, there are now extensive observations of molecules. Radio observations show absorption or emission by almost 50 molecules, a few of which radiate by maser action. Ultraviolet spectra of stars especially those obtained by the Copernicus satellite (Morton *Phil. Trans. R. Soc.* **A279**, 299; 1975) show

absorption by the  $H_2$  molecule. The abundance of interstellar  $H_2$  molecules can also be estimated from the radio observations of carbon monoxide. It is now plain that molecules are important in two ways. Many different molecules are observed in the clouds of gas that seem to be condensing into stars and the total mass of hydrogen as molecules and atoms is much greater than that as atoms alone.

Are molecules as important in stars? Clearly they are significant in the outer layers of cool stars. Now, in this issue of *Nature* (page 326) Jordan *et al.* describe their identification of  $H_2$  in the ultraviolet spectra of sunspots. The lines are in emission between 120 and 160 nm, and only a few of the possible lines of the  $H_2$  spectrum in that range are observed. Jordan and others show that the observed lines are just those to be expected if a particular level is preferentially populated by the absorption of the Lyman- $\alpha$  radiation of atomic hydrogen, which is of course very intense in the solar atmosphere. Selective fluorescence, as this process is, is of course familiar in laboratory studies. It is also known in astrophysics.

The spectra of molecules in comets are fluorescent spectra excited by sunlight, but again, not all possible lines occur. As was shown by Swings, the reason is that some energy levels are not excited because the frequencies of the transitions by which they would be excited from the ground state coincide with strong Fraunhofer lines in the solar spectrum. That is the reverse of the present case, where the upper level in the  $H_2$  spectrum is populated by absorption of a single line, Lyman- $\alpha$ ,

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molecules, predissociation phenomena may be observed in the spectra. The advent of synchrotron sources for ultraviolet spectroscopy makes it easier to investigate molecular spectra in the far ultraviolet, and prompted by the increasing astrophysical interest in molecules, it may be that there is an

area of physics here ripe for development. Thus the study of Jordan and others calls attention to these aspects of molecular astrophysics—the role of molecules in stellar atmospheres, the formation of molecular lines in interstellar formation and the expanding interest in laboratory investigations. □

## How should earthquakes be quantified?

*from a Correspondent*

At the meeting of the International Association of Seismology and Physics of the Earth's Interior (IASPEI) held in Durham on 9-19 August, 1977, a special workshop on the subject of Quantification of Earthquakes was organised by Professor K. Aki (Massachusetts Institute of Technology).

MUCH of the discussion in this workshop was concentrated on the concept of seismological magnitude, the oldest and most widely used measure of the size of earthquakes. Although magnitude has been an extremely useful phenomenological tool, recent advances in seismic source theory have convinced many seismologists that it is, if not obsolete, at least in need of revision and redefinition. There seems to be no agreement about how magnitude can be related to the physics of earthquakes or even about what it is that magnitude is intended to measure.

Also at Durham, the Subcommittee on Magnitude of the Commission on Practice met and wrestled with the problem of organising and simplifying the profusion of differing magnitude scales in current use. This task is almost impossibly difficult, due to variations in instrument design, wave types used, seismogram-reading practices, frequency and distance correction methods, and nomenclature. The situation has become so complex that even to catalogue current practices has required a major effort and has resulted in a document of impressive size. If the Subcommittee succeeds even partially in reducing the confusion and in standardising practices, it will have performed a valuable service.

Seismological magnitude was originally developed by Gutenberg and Richter in the 1930s as a heuristic tool for studying Southern California earthquakes. Before then, statistical studies of earthquake occurrence had made no distinction between large and small events, and often produced very misleading results. Magnitude was defined as proportional to the logarithm of the maximum excursion produced on a Wood-Anderson torsion seismograph

situated 100 kilometres from the epicentre, with magnitude 0 set at a level near the smallest detectable earthquakes. (Magnitudes down to about -3 are nowadays reported in micro-earthquake studies.) In this definition, Gutenberg and Richter were influenced considerably by the stellar magnitude scale in astronomy, from which seismological magnitude differs in two respects. First, and most trivially, the scales run in opposite directions: bright stars have small magnitude numbers, but great earthquakes have large ones. More importantly, seismological magnitude is intended to measure only the size of earthquakes, while astronomical magnitudes are of two varieties: absolute, which measures the total radiation from stars, and visual, which measures their brightness as seen (usually) from the Earth. The closest thing seismology has to visual magnitude is intensity, which is a measure of the strength of ground shaking in the epicentral region. Seismological intensity, however, is not based on any kind of measurement, but rather on human judgement applied to the social and engineering effects produced. It does not lend itself to quantitative interpretation, and in fact is usually expressed in Roman numerals to discourage any such attempt. As strong-motion seismographs become more widely distributed, it will become possible to place intensity on a more objective foundation. A first step in this direction might be to establish an instrumental scale for intensities below the threshold of perceptibility, where seismographic data are plentiful.

Gutenberg and Richter themselves seem to have been surprised at the great success of the magnitude concept, much of which no doubt stems from the fact that the range of magnitudes is enormous, so that even an imprecise scale is still quite useful. Detectable earthquakes range over more than 11 orders of magnitude, while even a gross blunder such as doubling the observed signal amplitude adds only 0.3 to the magnitude. By now however, our understanding of earthquake mechanisms has advanced to the point where

several physical attributes can be determined, at least approximately, from the radiated waves (such as source dimensions, displacement dislocation and stress drop). The relation between any of these quantities and magnitude is purely statistical, and subject to a great deal of scatter. (There is a case in California of a magnitude 3 earthquake with a fault length usually typical of magnitude 6 events.) It has become clear that several numbers are needed to describe an earthquake's size, and that magnitude is not one of them.

One of the most serious deficiencies of magnitude as it is now defined is the phenomenon of saturation. To determine the size of an earthquake, it is necessary to use waves longer than the spatial dimensions of the source. Otherwise, one measures only the radiation from a small part of the total source region at each instant. As a result the magnitude measured in any given instrumental pass-band has a practical upper limit. For 'short-period' (~1 s) waves this limit is around magnitude 7, while at 20 s it is about 8.5. If magnitude is based on the longest observable waves, then values range at least as high as 10 (for the 1960 Chilean earthquake). One solution to the saturation problem is to use waves longer than the source dimensions (how long this may be will vary from event to event). The quantity so measured is referred to as 'seismic moment', and its use has spread rapidly since its introduction some 10 years ago. It has the additional advantage of being subject to a simple physical interpretation: for a shear fault, for example, it is the product of the rigidity modulus, the fault area, and the mean dislocation across the fault. Seismic moment suffers, however, from being based only on low-frequency motions. In many contexts, such as that of hazard evaluation, high-frequency motions are more important.

Another related limitation of magnitude is the fact that it is based on instantaneous measurements. Signals of the same amplitude, whether they last for 1 cycle or 100, always lead to equal magnitudes, though they certainly do not correspond to similar source processes or hazards. Recently, local earthquake magnitude scales based on signal duration, or 'coda length', have been developed and are used widely in California, but they have not yet been given a sound theoretical foundation; the coda length observed is primarily a wave-propagation (scattering), rather than a source effect and may be subject to large variations with seismic region and focal depth. Coda-length magnitude has so far been used more to avoid problems of instrument saturation than to study the actual duration of earthquakes.

Considerable attention in the workshop was given to a generalisation of seismic moment, the 'seismic moment tensor.' This representation of seismic sources, which was suggested by Gilbert in 1970 (*Geophys. J. R. astr. Soc.* **22**, 223) and has recently been justified rigorously by Backus and Mulcahy (*Geophys. J. R. astr. Soc.* **46**, 341; and **47**, 301), promises to narrow the gap between observational seismology and the theory of earthquakes. The problem of understanding the earthquake process is one of the most difficult in mathematical physics, involving the elastic and anelastic behaviour, chemistry, and thermodynamics of rocks, and their interaction with the dynamic stress field. Seismologists are still far from solving this problem, and are likely to remain so for some time. When earthquakes are better understood, it may be possible to 'invert' seismic observations to derive event parameters, but meanwhile phenomenological descriptions will be needed, if only to catalogue observations we do not yet understand. This need explains much of the success of the magnitude concept, as well as the promise of the moment-tensor formulation. Backus and Mulcahy have shown that a unique reconstruction of the elastodynamic source process cannot, even in theory, be made from the information carried by the radiated waves; different physical sources can excite identical waves. What can be determined is the moment-tensor density, as a function of position and time, and any indigenous source can be described this way. To go further, unjustifiable assumptions must be made about the failure mechanism. The use of the moment-tensor representation, in fact, greatly simplifies the determination of source parameters, because the wave field depends linearly on the moment-tensor components, so the methods of linear inverse theory can be used. As a practical matter, this transformation of a non-linear inverse problem into a linear one enormously simplifies computations. The moment-tensor representation thus has the three advantages of being completely general, of being the most detailed source description which does not depend on a detailed model of the failure process, and of being computationally convenient.

It seems certain that moment tensors will prove to be powerful tools for advancing our understanding of the earthquake process. Agencies producing routine earthquake catalogues, such as the US Geological Survey and the International Seismological Centre, will probably soon begin to report moment tensors for large earthquakes, and these may someday replace magnitudes as the standard measure of earthquake size. In any event, though, seismo-

logical magnitude will be with us for some time, and its ultimate replacement or modification will occur because of scientific advances, not the reports and resolutions of official committees. □

## New earthquake magnitude scale

from Peter J. Smith

ALTHOUGH more than a million earthquakes occur each year, most of the energy released comes from the very few very large ones. There is some point, therefore, in being able to determine the seismic energy from very large earthquakes with reasonable accuracy. Unfortunately, there are difficulties in doing so. It is usual to estimate seismic wave energy ( $E$ ) from an earthquake's surface wave magnitude ( $M_s$ ) using the Gutenberg-Richter equation  $\log E = 1.5 M_s + 11.8$  or very similar empirical relationships derived by others (most notably Båth). The problem is, however, that for a great earthquake, which in this context means one with a rupture length of more than 100 km, the measured  $M_s$  is suspect.

The reason is that  $M_s$  is conventionally determined using surface waves with a period of about 20 s or by empirical conversion from a body wave magnitude derived from waves with even shorter periods. But when the rupture length of an earthquake exceeds the wavelength of the seismic waves used in the magnitude determination, the measured magnitude no longer reflects the entire rupture process; and there is then little correlation between  $M_s$  and rupture length. So the  $M_s$  scale is subject to 'saturation' at its upper end, the result of which is that for great earthquakes the measured magnitude and the energy estimated from it may be inaccurate.

In an attempt to circumvent this difficulty, which has been known for some time, Kanamori (*J. geophys. Res.* **82**, 2981; 1977) has now adopted a different approach to the calculation of the energy released by a great earthquake, an approach which leads incidentally to a more appropriate magnitude scale for such events. He thus begins not with magnitude but with seismic moment, a static source parameter defined as the product of the rigidity of the rock in the fault zone, the average slip over the fault surface and the area of the fault slip.

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Seismic moment ( $M_0$ ) is essentially a measure of the overall deformation at the earthquake source, but it can also be related to the elastic strain energy ( $W$ ) released by the earthquake. It turns out that if all the built-up stress is removed by an earthquake,  $W = W_0 \sim M_0/2 \times 10^4$ . If the stress drop is not complete the expression for  $W$  becomes more complicated and the calculated  $W_0$  gives only the minimum strain energy release. However, the situation is simplified if it be assumed, with Orowan (*Geol. Soc. Amer. Mem.* **79**, 323; 1960), that the stress remaining after the earthquake is equal to the frictional stress during faulting. In this case  $W_0$  is not only the minimum estimate of  $W$  but is also equal to the seismic wave energy ( $E$ ).

It is not clear whether or not the stress drop during an earthquake is complete; there is evidence to support either view. But there is also evidence from several earthquake studies to support the validity of Orowan's condition, which, if accepted, allows  $E$  to be determined from  $W_0$  and hence from  $M_0$ , irrespective of whether  $W_0$  is the actual or the minimum strain energy release. As for  $M_0$  itself, this quantity has been determined directly for many of the great earthquakes that have occurred since 1904, for others it may be estimated from the area of the fault plane or by other means, and for a few it may no longer be estimated at all. But whether  $M_0$  is known, by whatever means, Kanamori has determined  $W_0$  and hence  $E$ . By putting these  $E$  values into the Gutenberg-Richter equation he has then calculated magnitudes, denoted generally by  $M_w$ . In other words,  $M_w$  represents a new magnitude scale for great earthquakes, determined not directly from wave amplitude but indirectly from seismic moment.

The result is interesting. For the period 1904-76 the event with the highest  $M_w$  (9.5) turns out to have been the Chilean earthquake of 1960 ( $M_s = 8.3$ ). This was followed by the Alaskan earthquake of 1964 ( $M_w = 9.2$ ,  $M_s = 8.4$ ), the Aleutian earthquake of 1957 ( $M_w = 9.1$ ,  $M_s = 8.3$ ) and the Kamchatka earthquake of 1952 ( $M_w = 9.0$ ,  $M_s = 8.3$ ). But  $M_w$  is not always greater than  $M_s$ . For the 1920 Kansu (China) shock, for example, an  $M_s$  of 8.5 becomes an  $M_w$  of 7.8. Moreover, for many earthquakes with a rupture length of about 100 km,  $M_s$  and  $M_w$  are in fairly good agreement, suggesting that for these and smaller events the  $M_s$  scale is entirely appropriate. This also means that the  $M_w$  scale connects smoothly to the  $M_s$  scale to which it becomes a natural continuation.

The  $M_w$  scale seems to be better for earthquakes because it removes the saturation inherent in the  $M_s$  scale.

But it also draws attention to some possibly significant relationships which either were not previously apparent or are different from those implied by the use of the  $M_s$  scale. For example, when  $W_0$  is plotted against time for the period 1920–76 (for which the data are almost complete) it becomes clear that the annual average for the period 1920–65 (within which  $W_0$  peaks) is more than an order of magnitude higher than that for the periods 1920–50 and 1965–76. This picture is quite different from that based on  $E$  calculated from  $M_s$ , which shows a steady decrease since the mid-1940s. The significance of this difference becomes clear with the discovery that the  $E$ -from- $M_s$  curve is very similar in shape to the corresponding time curve of the number ( $N$ ) of earthquakes with  $M_s \geq 7.0$ . Since the  $N$  curve is heavily biased in favour of moderate-to-large earthquakes, the proportion of events

with  $M_s > 8.0$  being small (3.3%), the implication is that the  $E$ -from- $M_s$  curve is also biased in favour of moderate-to-large earthquakes, indicating that for great earthquakes the seismic wave energy calculated from  $M_s$  is indeed underestimated.

This being the case, it becomes clear from a comparison of the  $W_0$  and  $E$ -from- $M_s$  curves that the number of, and the wave energy radiated by, moderate-to-large earthquakes decreased sharply just when the energy from great earthquakes increased sharply. Was this just a coincidence or was there a causal link there somewhere? The evidence suggests the latter. As Kanamori demonstrates, there is a remarkable correlation between the  $W_0$  curve and the curve of Chandler Wobble amplitude, with  $W_0$  moving in the same way as, but slightly behind, the amplitude.

One possible explanation for this correlation is that an increase in Chandler Wobble amplitude, brought about by external (for example, atmospheric) forces, triggers worldwide seismic activity and accelerates plate motions, eventually leading to great plate-decoupling earthquakes which so decrease intraplate and interplate stresses that moderate-to-large earthquake activity declines. On the other hand, the activity of great earthquakes could produce the Chandler Wobble, although this seems to be less likely. Then a third possibility is that the Chandler Wobble and great earthquakes have a common cause such as perturbations in the Earth's rotation. Whatever the explanation may be, the ancient controversy over the precise connection, if any, between earthquakes and Chandler Wobble has evidently been revived. □

## New look for Loess Commission

from I. J. Smalley

Commission 4 of the International Union of Quaternary Research (INQUA) met at Birmingham University on 16 and 23 August, 1977. The scope of the Loess Commission is to be expanded, and practical aspects of loess investigation will be emphasised.

LOESS is essentially a silty soil, widely believed to have been deposited by the wind and is of particular interest as the soil on which some of the earliest agriculture flourished. The aims of the Loess Commission have been to produce a map showing the distribution of loess in Europe and to untangle some of the stratigraphic problems presented by the thick loess sequences of central Europe. It was formed as a subcommission at the 1961 INQUA meeting in Warsaw and upgraded to full commission status at the 1969 Paris meeting. Julius Fink (Austria) was its first president and he has steered the commission to the virtual completion of its initial tasks (see *Eiszeit. u. Gegenwart* **27**, 220; 1976). At Birmingham, progress on the loess map was reported by G. Haase (DDR); the western sheet is virtually complete and proof prints should be available by the end of 1977. The eastern sheet has been delayed by the lack of a suitable topographic base but with the help of I. P. Gerasimov (USSR) this difficulty has now been overcome. Details of the map scales and the data presented are given in a paper by Haase, Ruske (DDR) and Fink which will appear soon in *Petermanns Geog. Mitt.*

Fink reported on field trips since the last congress (Christchurch 1973); visits have been made to Germany (BRD, 1974), France (1975) and the Soviet Union (Ukraine and Moldavia, 1976). Now that the European problems have been tackled the commission is looking east to the Asian loesses and the 1976 trip to Soviet territory represents the first tentative

move in that direction. The commission will also turn to the study of more practical aspects of loess and give some emphasis to irrigation problems and engineering and economic topics. New INQUA bye-laws require sweeping changes in the commission membership and advantage will be taken of this to reflect new interests (both topical and regional). The new president is Marton Pecs (Hungary), Director of the Geographical Institute of the Academy of Sciences in Budapest; vice-president, B. Frenzel (BRD); secretary, O. Fränze (BRD); and a group representing Europe: J-P. Lautridou (France), J. Fink (Austria), J. Macoun (Czechoslovakia), A. E. Dodonov (USSR) and I. J. Smalley (UK). R. V. Ruhe (USA) will co-ordinate activities in North America and J. M. Bowler (Australia) will do the same for the Australasian and Pacific regions; there are eighteen corresponding members.

J. M. Bowler reported on the contacts between Australia and China. A group from the Australian National University has visited the classic loess

regions of north China and returned with samples and Chinese loess literature. Important papers will be translated and published in English. In particular the major works by Liu Tung-sheng and his collaborators will be made available. Chinese investigators have visited Australia to look at aeolian sediments in the central regions. There is no loess as such in Australia but the aeolian sediments have enough features in common to make comparative studies worthwhile. The contact at present is established at governmental level but the commission hopes to participate in studies of one of the world's most fascinating and still problematical (see *News and Views* **267**, 484; 1977) loess deposits.

Pecs rounded the meeting off with a personal view of new aims for the commission. He stressed the need for research into practical problems connected with loess and proposed that in the next phase of the commission's activities there should be some emphasis on foundation problems and related engineering topics, on problems of landscape deterioration in loess areas, and on problems of forecasting and planning for land use. Traditional activities should not be neglected however and it might be possible to investigate certain loess stratotypes very thoroughly—perhaps on an international basis. He proposed that the section at Paks in Hungary might receive the same intense study as that organised by Fink at Krems in Austria.

The next loess discussion will probably be in Jerusalem at the Sedimentological Congress in July 1978. D. H. Yaalon (Israel) will prove to doubters that desert loess exists. □

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# review article

## Evolution of ageing

T. B. L. Kirkwood\*

*An evolutionary view of ageing suggests that mortality may be due to an energy-saving strategy of reduced error regulation in somatic cells. This supports Orgel's 'error catastrophe' hypothesis and offers a new basis for the study of normal and abnormal ageing syndromes and of apparently immortal transformed cell lines.*

THE question of why we and most other higher animals age is not trivial since many organisms, notably higher plants, live and propagate indefinitely. Perhaps because we are so familiar with ageing it is dangerously easy to fall into circular argument such as that ageing is a mechanism for ridding a population of old and worn-out individuals who would otherwise compete for resources with younger and fitter ones<sup>1</sup>. This makes sense if, for example, we assume individuals to have a defined reproductive lifespan, but is circular since the cessation of reproduction is itself a phenomenon of ageing. In this paper I show that by adopting an evolutionary point of view it is possible to relate Orgel's theory<sup>2</sup> that ageing is the result of a progressive breakdown in accuracy in protein synthesis to recent theoretical and experimental work on the senescence of diploid human fibroblasts, and I suggest a new reason why we may expect the error theory of ageing to be correct.

In discussing the evolution of error regulatory mechanisms it is appropriate to begin with brief consideration of the emergence of the earliest life-forms. The fate of the first replicating organic molecule would have depended on the speed and accuracy with which it generated 'daughters'. Subject to a continual risk of disruption by chemical and physical agents in the environment the lifetime of any relatively complex molecule must have been strictly limited. In addition primitive replication was probably highly inaccurate so only a relatively small proportion of copies would have been viable. To avoid extinction a replicator needed to produce at least one viable copy before being disrupted and even so there remained a definite chance of extinction which decreased inversely with the expected number of descendants per generation. It is, therefore, highly unlikely that life began all at once in a particular place at a particular time. Much more plausibly large numbers of colonies of different replicators were appearing and disappearing over the same period of time. Gradually, and inevitably, the colonies became larger as the faster and more accurate replicators outlasted the others. The mean levels of accuracy and speed of replication continued to rise while the freely available raw material for biosynthesis was depleted, leading to intensive interspecies competition and strong selection for catalytic activity and hence, for example, to the evolution of organised cells capable of binary fission.

### Coping with errors

At a fundamental level evolutionary survival is the preservation of a dynamic balance between information, or

order, and entropy, or disorder. Indeed, given the capacity for replication, neo-Darwinist natural selection may be seen as the direct result of interaction between the information stored in living organisms and the entropy of their environment. The diversity of present-day species testifies to the wide range of evolutionary strategies that are effective for survival, and it is my intent in this paper to demonstrate why ageing should be one of these.

A vital manifestation of entropy is the error inherent in all processes of macromolecular information transfer within living cells. To sustain the prospect of further evolutionary change and so improve its chance of ultimate survival an organism must make occasional copying errors. Too many mistakes would be a serious handicap so what is needed is a compromise, a tolerable level of error sufficient to allow necessary change. Given enough time natural selection ensures that organisms with optimal error levels will dominate. The presence of any error at all, however, poses interesting problems for our understanding of molecular biology.

Orgel<sup>2</sup> pointed out that cellular proteins could be divided roughly into two sets, those involved in the transfer of information from DNA, in replication and in protein synthesis, and those involved in other cell functions. The presence of a low level of error in the synthesis of the second set of proteins would have relatively little effect on cell viability. But erroneous synthesis of some of the first set might have serious consequences since abnormal proteins that retained activity but had reduced specificity could produce further defective proteins and so generate a positive feedback of errors leading to a progressive decline in the accuracy of the protein-synthesising machinery and ultimately to a lethal 'error catastrophe'. Orgel subsequently revised<sup>3</sup> his original suggestion that the error frequency must increase exponentially and showed that depending on the amount of error feedback allowed, stability of translation may be achieved despite the presence of error. In some circumstances, however, error catastrophe would still occur. Hoffman<sup>4</sup> has proposed a more detailed model of the feedback of errors in the protein-synthesising machinery and concluded that translation is too stable to allow 'error catastrophe' to play any real part in present-day organisms. He supposes the translation apparatus to comprise a set of polypeptide 'adaptors' responsible for inserting amino-acids into a peptide chain according to the sequence specified by the DNA. The average accuracy of the  $i$ th generation of adaptors (assuming these to be in some sense discrete generations)—that is the probability that an adaptor of the  $i$ th generation inserts the correct amino-acid—is denoted by  $q_i$ . Then what matters is the form of the functional relationship between  $q_i$  and  $q_{i-1}$ . If  $q_i > q_{i-1}$  the error level

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## Complete accuracy

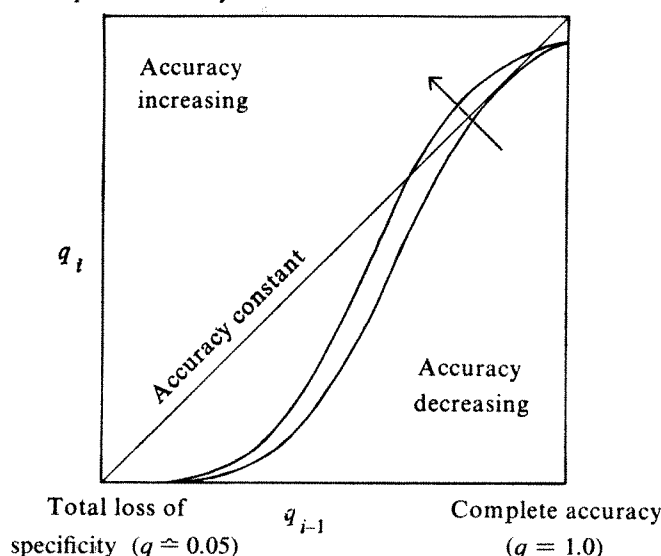


Fig. 1 A simplified model<sup>4,5</sup> of the feedback of errors in the translation apparatus assumes a set of polypeptide "adaptors" to be responsible for inserting the specified amino acids into peptide chains. The curves illustrate the form of relationship between the average accuracy,  $q_i$ , of the generation  $i$  adaptors and the average accuracy,  $q_{i-1}$ , of the immediately preceding generation. The arrow indicates the direction of displacement of the curve as the average proportion ( $R$ ) of activity retained by erroneous adaptors decreases.

decreases while if  $q_i < q_{i-1}$  it increases. According to Hoffman the dependence of  $q_i$  on  $q_{i-1}$  is S-shaped with a region where  $q_i > q_{i-1}$  for any plausible present-day apparatus. The presence of this stable region forms the basis of Hoffman's conclusion that 'error catastrophe' is not relevant to present-day organisms. Kirkwood and Holliday<sup>6</sup> point out, however, that Hoffman's model requires that the activity of an erroneous adaptor be reduced to a level so low that feedback of errors is virtually prohibited and argue that a considerable body of biological evidence does not support this assumption. They define a parameter  $R$  that represents the proportion of activity retained by erroneous adaptors and show that the value of  $R$  determines whether or not stability is attained (see Fig. 1), low values of  $R$  resulting in stability (Hoffman's model is equivalent to the case where  $R$  is fixed and very small). The introduction of this parameter into the model is of great importance since it allows a degree of freedom on which evolution may work. For example, the development of a 'scavenging' system that would degrade erroneous protein would reduce the value of  $R$ .

It should immediately be recognised that this model is a great oversimplification and cannot be viewed as anything more than a broadly descriptive account of the possibilities of error catastrophe or stability. It does, nevertheless, serve to illuminate the general qualitative features that can be expected of the feedback of errors in the protein-synthesising machinery and it forms a useful basis for discussing their relevance to the evolution of present-day organisms.

As primitive cells evolved, each containing its own translation apparatus, some stability of error must have been achieved. Error catastrophes may, of course, have been initiated in some daughter cells but the expected number of 'catastrophe-free' offspring per generation must have been greater than one. Selection would have favoured those cells with the highest proportion of 'catastrophe-free' offspring and so increased the level of stability.

This can be understood quite easily in terms of the Kirkwood/Holliday model. As  $R$  (the proportion of activity retained by erroneous adaptors) decreases the S-shaped

curve relating to  $q_i$  to  $q_{i-1}$  moves steadily upwards and to the left. Thus an  $R$ -value can be chosen such that the curve just touches the line  $q_i = q_{i-1}$  giving a point of precarious balance. A slight further decrease in  $R$  produces a small stable region ( $q_i > q_{i-1}$ ). Random fluctuation in  $q$ , however, may allow a jump across the stable region into incipient error catastrophe. Decreasing  $R$  decreases the chance of such a jump. Thus we can see that selective pressure continues to reduce  $R$  until the chance of jumping across the stable region becomes very small. This would have been particularly true for organisms exposed to environments where the risk of externally introduced errors was high.

On this basis we can expect to find that in present-day organisms the stability of translation will be very high. At this point, however, it is important to draw a distinction between unicellular and multicellular organisms. In discussing ageing our attention is confined to the latter in which the cells may be grouped as somatic cells which do not contribute information to succeeding generations and germ line cells which do. We shall see that there are good reasons why an organism should have evolved a different approach to error regulation in these two categories of cells and that these are sufficient to explain why we age.

### The phenomenon of ageing

Since Hayflick and Moorhead<sup>6</sup> gave the first clear demonstration that human fibroblast cells have a finite lifespan *in vitro* these cells have been studied as a possible model for ageing *in vivo*. Fibroblast lifespan has been shown to be determined largely by cell generations and not by chronological time<sup>7</sup>, and Martin *et al.*<sup>8</sup> have proved that the finite division potential is related to ageing *in vivo* by demonstrating that culture lifespan declines with donor age. A similar conclusion was indicated by the finding that cells from patients with diseases of premature ageing showed reduced division potential.

Senescent fibroblasts are visibly sick and cannot compete with young cells. It is therefore surprising that cellular selection does not act against the senescent cells leaving the culture permanently viable. The most satisfactory explanation is that cells become 'committed' to senescence while still outwardly healthy and that a number of cell generations then elapse before the death of the resultant clone. A model on these lines has been shown to explain the finite lifespan of human fibroblasts in culture<sup>9</sup>. This model is very simple in structure. It is assumed that any cell not yet committed to senescence gives rise to committed daughter cells with some probability  $P$  for each daughter and that an incubation period of  $M$  cell generations is required before death. If  $P \geq 0.5$  the expected number of uncommitted daughters per uncommitted cell is  $\leq 1$ , so mortality is inevitable. If  $P < 0.5$  the uncommitted cells can in principle increase in number. In practice, however, the culture size is strictly limited and surplus cells are discarded with the result that the uncommitted cells are lost 'by dilution' unless the culture size is very large (routine cultures contain  $10^6$ – $10^7$  cells). Experimental results<sup>10</sup> strongly support this model and suggest that  $P \approx 0.275$  and  $M \approx 55$ – $60$ .

The conclusion we might be tempted to draw from the observed mortality of cell cultures, namely that ageing *in vivo* is the result of the body's cells simply running out of division potential, is denied by the data of Martin *et al.*<sup>8</sup> which show that mean culture lifespan (population doublings)  $\approx 42 - 0.2 \times$  donor age so that cells from even 90–100-yr-old individuals are capable on average of 20–25 population doublings. A specific feature of the commitment model is that it predicts a sharp fall in the cell population growth rate as the first committed cells reach the end of their incubation period and die (see Fig. 2) since these constitute approximately a quarter of the population. For normal cell cultures the change of growth rate may be

expected 20–25 population doublings before the end of the culture lifespan. Tissue in old people may therefore be experiencing this fall in growth rate. This conclusion is supported by the estimated value of 55–60 cell generations for the incubation period which allows  $\approx 10^{17}$  cells to be produced from a single cell before the change in growth rate would occur, a number reasonably close to the total number of cells likely to be required in a normal human lifetime. Ageing is a complicated process and it may be a mistake to seek too simple an explanation. It is, however, possible that the relatively sudden loss of a substantial proportion of cell replicative potential may account for the physical emaciation and loss of homeostasis associated with senile decay.

### Why grow old?

The commitment model closely resembles the model for the feedback of error in the protein synthesising machinery if we equate  $P$  with chance of jumping from a stable to an unstable state and  $M$  with the metabolic period required for an error catastrophe to result. Fifty-five to sixty cell generations may seem surprisingly long in this context, but  $q_i$  initially changes slowly and it can be shown that many generations of adaptors may have to elapse before  $q_i$  falls very far.

Experiments show that increasing the error frequency reduces the lifespan of human fibroblasts<sup>11</sup> and that old cells contain higher levels of erroneous functional and translational enzymes than young cells<sup>11,12</sup>. A general error catastrophe thus offers a plausible explanation for the ageing of human fibroblasts. That it offers a likely explanation may be seen from the following argument.

As Hamilton and others have recently emphasised (see ref. 13) natural selection must operate at the level of the replicating unit, the gene. To maximise their chance of survival genes must always replicate between succeeding generations as rapidly and accurately as allowed by their environment. Accuracy can only be achieved through the

expenditure of energy, either by direct proof-reading, kinetic proof-reading as proposed by Hopfield<sup>14</sup>, or by destroying erroneous products. The latter may occur either at a molecular level by scavenging for erroneous protein or at a cellular level by means of a 'suicide' protein that kills the cell when the error level begins to rise<sup>2,15</sup>. Accuracy in the germ line is vital for gene survival but a high level of accuracy in somatic cells may be a luxury our genes do better to forego. Ageing may, therefore, be the result of an energy-saving switching off of the mechanisms responsible for high accuracy in the translation apparatus at or around the time of differentiation of somatic cells from the germ line. This may leave the somatic cells in a relatively unstable state where a small random increase in errors commits them to an eventual error catastrophe.

This strategy is evolutionarily stable in the sense defined by Maynard Smith and Price<sup>16</sup> provided that the risk to the individual of accidental death is not too low since the alternative of maintaining the high accuracy in all cells which might allow immortality would carry the penalty of an increased energy requirement. This penalty would mean that, other things being equal, an individual would grow and reproduce more slowly or would be subject to a greater risk of starvation or accidental death while seeking food.

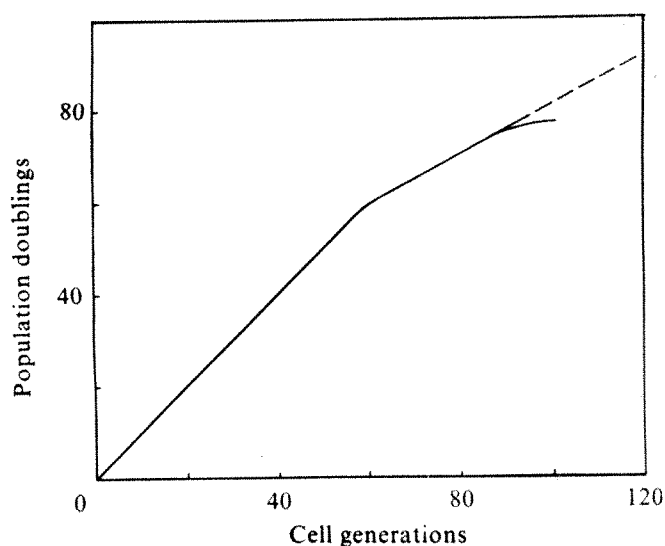
### Conclusions and implications

There are three requirements for a satisfactory theory of ageing. It must be theoretically plausible, it must be supported by experimental evidence, and it must make evolutionary sense. The general error theory satisfies all these requirements. The main rival theory suggests that ageing is genetically programmed in much the same way as morphogenesis. Although this is theoretically plausible no specific model has been proposed that is amenable to experimental test and no convincing argument has been put forward to favour the evolution of such a programme. A further theory<sup>17</sup> suggests that ageing is the result of natural selection delaying the time of onset of deleterious genes, so that senescence and death result from the accumulation of late-acting harmful genes. This well-argued theory is, however, either circular or incomplete as it presupposes ageing in a general sense since the time of action of a gene during adulthood is determined not by chronological time but by its biochemical environment. If the time-keeping process is a genetic 'clock' the theory is identical with the programme theory. If it is an increase of errors it may be incorporated as a part of the general error theory. A third possibility, that the genes are triggered by the accumulation (or depletion) of some otherwise innocuous substance, would need to be specifically defined and tested before it could be seriously entertained.

If the argument presented here is correct, we may hope to understand some of the many aspects of ageing by detailed study of the processes of error regulation. We may examine how different species have adapted these processes to meet their individual needs and, in particular, we may hope to gain insight into the abnormalities represented by diseases of premature ageing and by the apparently immortal transformed cells of malignant tumours. It may, for example, be possible that oncogenic viruses interfere with these processes to enhance the accuracy of their own replication in a way that restores some error-committed cells to stability. Such a return to a quasi-germ-line state might explain the reappearance of foetal proteins in malignant tissue. We should remember too that our ageing processes evolved at a time when our average lifespan was considerably shorter, just long enough in fact for us to fulfil our basic evolutionary role of bearing children and nurturing them to reproductive age. In modern technological societies the majority of us reach old age and mortality need not necessarily be evolutionarily stable.

I am very grateful to Dr R. Holliday for continued help

**Fig. 2** Predicted fall in cell population growth rate according to a model of the commitment to senescence of diploid human fibroblasts<sup>9</sup>. Cells not yet committed are assumed to give rise to committed daughter cells with probability  $P$  ( $\approx 0.275$ ) for each daughter.  $M$  ( $\approx 55$ –60) cell generations after commitment the resultant clone is assumed to die. An initially uncommitted population doubles with each cell generation until, after  $M$  generations, the first deaths occur and the population growth rate falls. During routine cell culture, when the population size is strictly limited, uncommitted cells are lost "by dilution" and the population dies out (solid curve). Very large cultures may, however, continue indefinitely at the reduced growth rate (broken line).



and encouragement with this work and to Professor J. Maynard Smith and Dr L. E. Orgel for discussion.

1. Weismann, A. in *Weismann on Heredity* (eds Poulton, E. B., Schönland, S. & Shipley, A. E.) 2nd edn, 23–42 (Oxford University Press, 1891).
2. Orgel, L. E. *Proc. natn. Acad. Sci. U.S.A.* **49**, 517–521 (1963).
3. Orgel, L. E. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1476 (1970).
4. Hoffman, G. W. *J. molec. Biol.* **86**, 349–362 (1974).
5. Kirkwood, T. B. L. & Holliday, R. *J. molec. Biol.* **97**, 257–265 (1975).
6. Hayflick, L. & Moorhead, P. S. *Expl Cell Res.* **25**, 585–621 (1961).
7. Littlefield, J. W. in *Variation, Senescence and Neoplasia in Cultured Somatic Cells*, 62–64 (Harvard University Press, Cambridge, Massachusetts, 1976).
8. Martin, G., Sprague, C. & Epstein, C. *Lab. Invest.* **23**, 86–92 (1970).
9. Kirkwood, T. B. L. & Holliday, R. *J. theor. Biol.* **53**, 481–496 (1975).
10. Holliday, R., Huscchtscha, L. I., Tarrant, G. M. & Kirkwood, T. B. L. *Science* (in the press).
11. Holliday, R. & Tarrant, G. M. *Nature* **238**, 26–30 (1972).
12. Linn, S., Kairis, M. & Holliday, R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2818–2822 (1976).
13. Dawkins, R. in *The Selfish Gene* 13–21 (Oxford University Press, 1976).
14. Hopfield, J. J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4135–4139 (1974).
15. Holliday, R. *Fedn Proc.* **34**, 51–55 (1975).
16. Maynard Smith, J. & Price, G. R. *Nature* **246**, 15–18 (1973).
17. Medawar, P. B. *An Unsolved Problem in Biology* (H. K. Lewis, London, 1952).

## articles

# Drift of the major continental blocks since the Devonian

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*Palaeomagnetic evidence indicates that the continents have been in a more-or-less continuous relative motion. At the end of the Palaeozoic there was a redistribution of the major continental blocks that occurred without the formation of new ocean between them. Wegener's Pangaea seems only to have lasted a few tens of millions of years.*

MAPS summarising the drift of the major continental blocks since the middle Devonian (–375 Myr ago) are presented here. They are based mainly on a global synthesis of the palaeomagnetic data<sup>1</sup>, including the most recent results from the USSR<sup>2</sup>. Reasonably reliable apparent polar wandering (APW) paths for the major continental blocks can now be drawn for the Middle Carboniferous (325 Myr ago) onwards, and are given in Figs 1 to 3. They have been obtained by first referring all the palaeomagnetic poles to a scale of Myr<sup>3</sup>. The oldest poles from each continental block were then grouped into an interval of 40 Myr duration, and an average calculated to obtain the oldest point. The limits of the interval were then moved forward by 10 Myr and a second average taken, and so on. Only every fourth value is independent. It would be desirable to use averages over a shorter interval, but as yet the data are neither numerous enough, nor sufficiently well spread in time. Calculations using 30 and 40 Myr intervals yielded almost identical mean values, but at 20 Myr more scatter was evident. The mean 95% errors are 6° (North America), 7° (Eurasia) and 7° (Gondwana).

The calculations were made assuming that the geomagnetic field averaged to an axial geocentric dipole (AGD). There is evidence that even when averaged over millions of years, the field may have had long-term non-dipole components<sup>4</sup> sufficient to cause polar errors of about 5°, and there are physical reasons why this might be so<sup>5</sup>. Analysis<sup>6</sup> of the geomagnetic field over the past 25 Myr yields no evidence for departures greater than about 5°. Indeed, it has recently been shown that during the Phanerozoic there is no significant departure of the field from that of an AGD within the errors in the palaeomagnetic determinations<sup>7</sup>. There is good agreement between the palaeomagnetically determined latitude and the palaeoclimatic evidence, and this supports the AGD assumption in a general

way<sup>8</sup>; for example, glaciations occur in Australia from the Middle-Carboniferous to the end of the Permian<sup>9</sup>, and their disappearance coincides with the motion of the pole away from Australia (Fig. 3). Finally, there is excellent internal agreement among Gondwana poles from localities spaced over 9,000 km, among poles from localities spread over 5,000 km in North America, and among poles spread over 6,000 km from Eurasia; note, for example, the high precision ( $k = 103$  to 406) of Gondwana poles in the interval 270 to 290 Myr ago from Argentina, Morocco, Madagascar, Tanzania and Australia (Table 1). It is unlikely that such internal agreement would have been observed within blocks that at the time were adjacent to one another, if the field really had had very large non-dipole components. Therefore there are grounds for supposing that the poles of Table 1 provide estimates of the geographical pole with an average accuracy of 6° or 7° ( $P = 0.05$ ).

Since the advent of plate tectonics, maps showing displacements of the continents have been made using evidence mainly from the oceans, information that extends back only to the early Jurassic. Such maps indicate that at about 200 Myr ago the continental crust was grouped into a single supercontinent<sup>10</sup>, referred to here as Pangaea A. Pangaea A is essentially Wegener's reconstruction<sup>11</sup>. But for how long, before 200 Myr ago, did Pangaea A exist? This may be studied by comparing the latitudes for reference localities that were once adjacent but are now far apart using the paths of Figs 1 to 3. If Pangaea A existed the latitudes should be the same. For the comparison between North America and Africa (Fig. 4) the reference locality used is midway between Cape Blanc (locality L 21°N, 19°W in the African frame) and Cape Hatteras (locality M 37°N, 74°W in the North American frame) using the most recent reconstruction<sup>12</sup> of Pangaea A;  $\Delta\lambda = \lambda_L - \lambda_M$ , and  $\lambda$  has been calculated from Table 1 using equation (9.1) of ref. 8. For the Lower Jurassic (190–160 Myr)  $\Delta\lambda$  is effectively zero, so that the palaeomagnetically determined latitudes agree with Pangaea A. Before that  $\Delta\lambda$  is positive and significant, suggesting that during the Permian and much of the Triassic, Africa had a more northerly position relative to North America than that indicated by Pangaea A. This result is independent of the choice of reference localities, and is essentially the same for other reconstructions<sup>13,14</sup>. This discrepancy, although not previously so well documented, has been known for many years, and

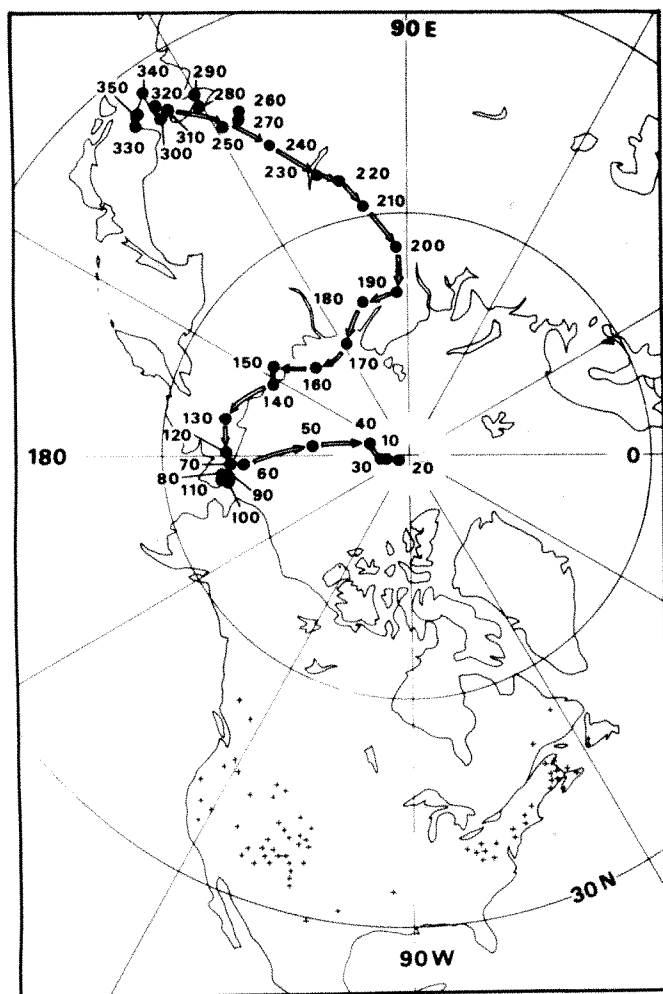


Fig. 1 Apparent polar wander (APW) relative to North America since the Devonian. Sampling localities are marked by crosses. Errors listed in Table 1.

several workers<sup>15-18</sup> have proposed that there was a large post-Triassic shear along Tethys (the Tethys twist<sup>17</sup>), that was supposed to account for the Alpine deformation<sup>16,17</sup>. This idea is no longer acceptable because the early Jurassic palaeomagnetic results are now known to be in excellent agreement with Pangaea A (Fig. 4); the motions needed to account for the discrepancy must have occurred before the Alpine Orogeny, and before the creation of the western part of Tethys in the late Triassic. For the comparison between North America and Europe the corresponding  $\Delta\lambda$  values using Orphan Knoll as the reference locality ( $P$  50°N, 47°W in the North American frame, and  $Q$  50°N, 17°W in the European frame  $\Delta\lambda = \lambda_P - \lambda_Q$ ) do not differ from zero in the Permian and lower Jurassic, but there is a significant departure of about 10° in the Triassic (Fig. 4). It has been proposed<sup>19</sup> that this is best explained by assuming that Eurasia moved southward with respect to North America during the lower and middle Triassic. Although this is probably the correct interpretation of the discrepancy itself, it alone does not explain the remarkable fact that later, in the lower Jurassic, the results are again in excellent agreement with Pangaea A (Fig. 4). Tectonic explanations for the discrepancies of Fig. 4 that are globally compatible with all the available palaeomagnetic data are now presented.

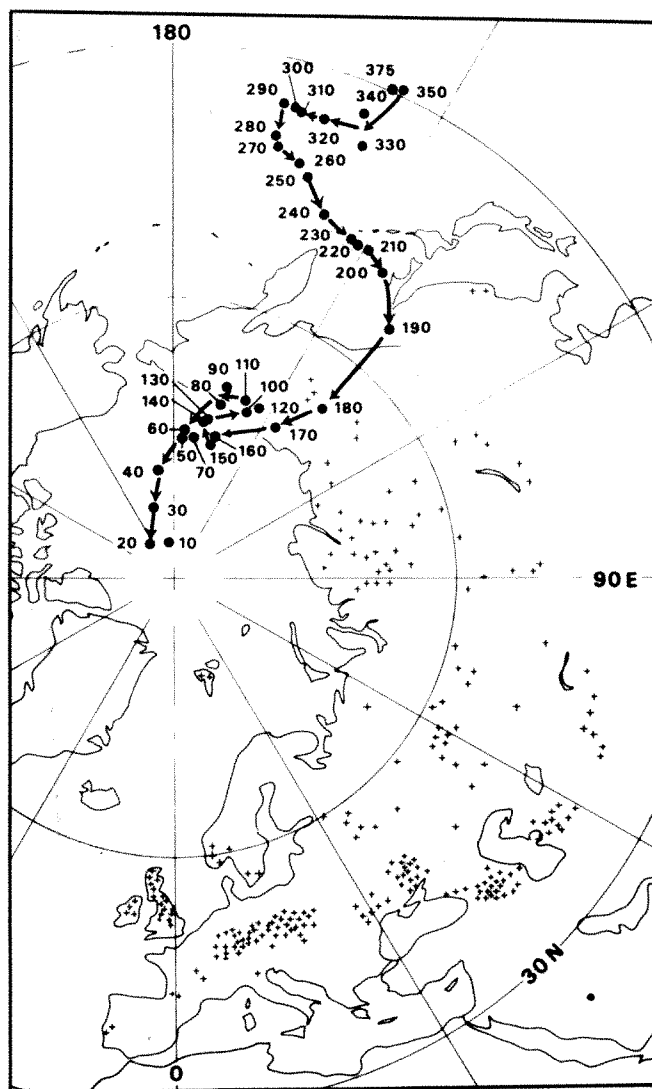
APW paths define the latitudes and azimuths but not the longitudes of continents. In order to obtain maps for post-Pangaea A time (200 Myr ago) the latitudes of the continents have been fixed palaeomagnetically, and their relative longitudes have, for the most part, been taken from plate tectonic reconstructions<sup>10,20-22</sup>. Maps for times before 200 Myr ago have

been made by working backwards, interval by interval, from Pangaea A, maintaining consistency with the palaeomagnetically determined latitudes and azimuths, but restricting relative motions to a minimum and disallowing major overlap between continents.

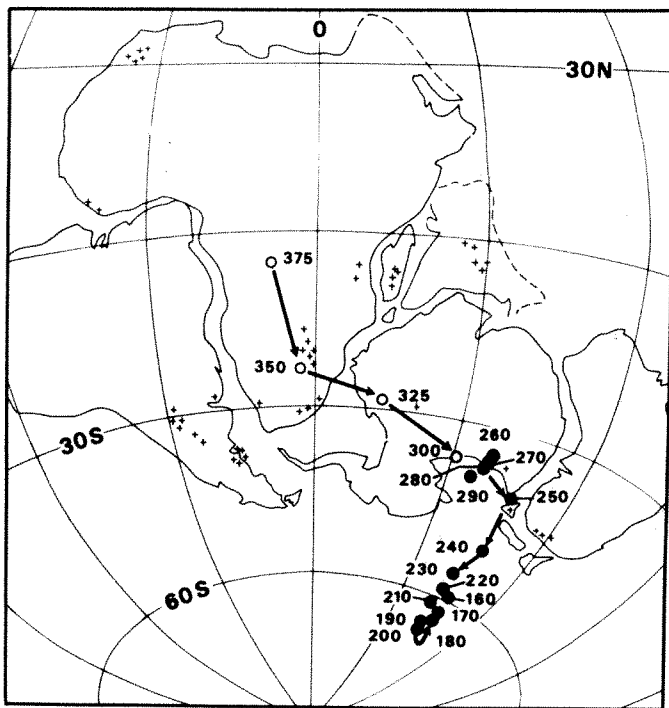
### Description at maps

The suggested reassembly for the mid-late Devonian (375 Myr ago) is shown in Fig. 5a. In the north there is a more-or-less coherent supercontinent, Laurasia, consisting of the Laurentia, Baltic-Russian and Siberian cratons and their bordering Early Palaeozoic foldbelts. Several authors<sup>27,28</sup> have argued that in the Devonian there was a wide ocean on the site of the Ural foldbelt. But, the palaeomagnetic poles from the Devonian of the Russian and Siberian platforms, are in reasonable agreement, and therefore it is assumed that there was no more than a narrow trough between them. The mean Devonian pole for the Russian platform is 32°N, 161°E,  $\alpha_{95} = 5^\circ$  (5.120), for the Dneister region it is 39°N, 161°E,  $\alpha_{95} = 7^\circ$  (5.130), for the Ural region it is 38°N, 165°E,  $\alpha_{95} = 9^\circ$  (5.177) and for the Siberian platform 24°N, 149°E,  $\alpha_{95} = 8^\circ$  (5.178). (The numbers in brackets refer to entries in ref. 1 where the bibliography and analytical details may be found.) Because of the longitude indeterminacy this solution is not a rigorous one, but only the simplest, which devolves from the constraint of minimum

Fig. 2 APW relative to northern Eurasia since the Devonian. See legend Fig. 1.







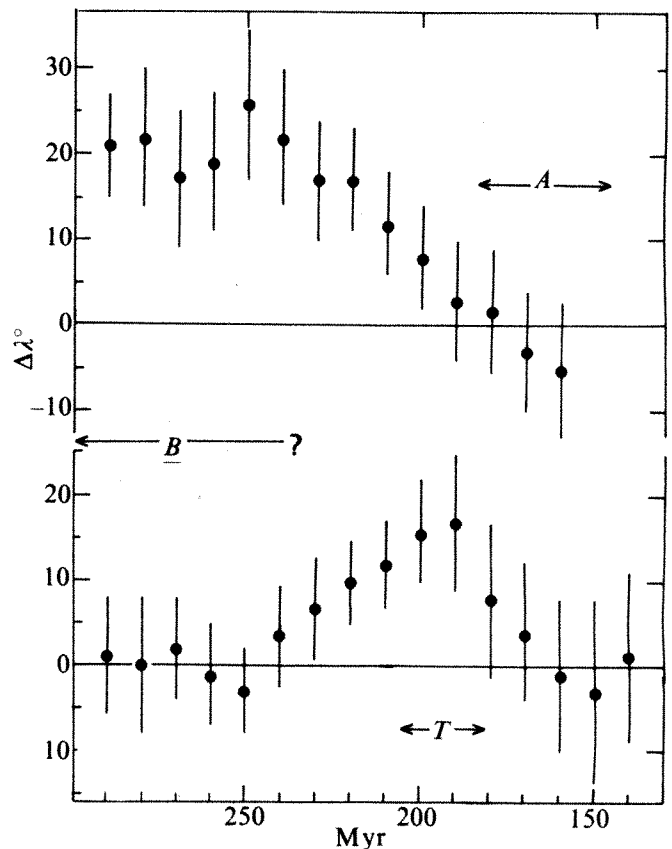
**Fig. 3** APW relative to Gondwana for the Late Palaeozoic and Early Mesozoic. ●, Are the poles of Table 1 and have been obtained by statistical analysis; ○, for the interval 375 to 325 Myr ago, are approximate poles, obtained by visual inspection of sparse data from Australia and South America only. This interpretation follows that given in refs 43, 45 and 46, and is speculative. Gondwana began to split in the Jurassic, and an average path cannot be obtained for later times. See legend Fig. 1.

motion. If the Urals are the site of a collision between the two platforms it seems more likely that the major movement occurred in the early rather than the late Palaeozoic. This problem is not of direct interest in the present context and will be considered again later (Morel and Irving, in preparation). In the late Devonian assembly of Fig. 5a Spitzbergen adjoins Ellesmere Island and Greenland<sup>23</sup>, and northern Alaska is rotated with respect to North America<sup>24,25</sup>. There is at present no palaeomagnetic information from the northern two-thirds of Alaska that would shed light on this speculation, but there is indirect evidence. In the Mid-Cretaceous (100 Myr ago) the pole positions relative to Eurasia and North America make it impossible to place them together without substantial overlap of their continental shelves (Figs 1 and 2). Alaska, or NE Siberia, or both have moved. Chukotsk<sup>26</sup>, with or without the Kolyma block (Irving and Geuer, in preparation), may have been continuous with northern Alaska. It is possible that all three elements (northern Alaska, Chukotsk and Kolyma) moved together, their tectonic juncture with Asia being the huge fault systems of the Khrebet Cherskogo. Alternatively, Kolyma may have been welded on to Asia separately<sup>34</sup>, and this is accepted here. The possible position of NE Siberia relative to northern Alaska is indicated by *H*? in Fig. 5. A further feature of Fig. 5a to *i* is an indentation in the northern border of Laurasia. This is the Sinus Borealis, or proto-Arctic, which closed in the early Cretaceous (see below). To the south of Laurasia there may have been an ocean about 2,000–3,000 km wide (see legend to Fig. 5). The southern continents are assembled into a supercontinent, Gondwana<sup>29</sup>.

In the zone of interaction between Laurasia and Gondwana, the positions of many crustal elements are uncertain. For example, Spain and central Europe may have been situated in position *B* or *C* (see ref. 30) of Fig. 5, and the USA south and east of the Appalachians could have occupied either positions

*A* or *B*. The best recent data<sup>31</sup> favour position *B* for southern France. The data from Spain are indecisive. Nor is very much known about the positions of Iran, China<sup>32</sup> and southern Indo-China. This paper is concerned with the major continental blocks and no detailed consideration of these questions is given.

At the Devonian–Carboniferous boundary, Laurasia and Gondwana are converging and they meet in the Mid-Carboniferous. The relative longitudes of Fig. 5c were chosen so as to minimise their subsequent relative motion. Africa is opposite



**Fig. 4** Latitude differences ( $\Delta\lambda$ ) for reference localities from North America and Gondwana (above), and North America and Europe (below), for the later Palaeozoic and Early Mesozoic using the reconstruction of Pangaea B<sup>12</sup>. See text for method of calculation. The errors at ( $P = 0.05$ ) are given. The approximate durations of Pangaea A and B are marked. *T* is the suggested time of opening of the western Tethys.

Europe, and South America opposite North America. This is referred to as Pangaea *B*. A similar reconstruction for the Permian has recently been given independently by Westphal<sup>34</sup>, and the earliest record of it is in ref. 9 (Fig. 10, 17). There are, however, no palaeomagnetic constraints on longitude, so Gondwana could have been further east, or it could have been situated west of North America. These optional configurations<sup>17,18</sup> are considered less likely because the subsequent motions needed to achieve the known late Jurassic configuration (Fig. 5i) would have been greater—a factor of two greater in the latter instance. In Gondwana the Samfrau<sup>29</sup> super-foldbelt develops. It extends for 6,000 km from the Tasman foldbelt, through the Ellsworth and Cape foldbelts to the central Andes. By the end of the Carboniferous the fit between Laurasia and Gondwana tightens, and apparently remains unchanged in the early Permian, although small adjustments, undetectable palaeomagnetically, could be occurring (Fig. 5d to *f*).

At the Permian-Triassic boundary the palaeomagnetically determined latitudes now allow Gondwana to rotate anticlockwise, and the transformation from Pangaea *B* to *A* to begin. This is made possible by a northward movement of North America<sup>19</sup> along a zone (*XM*), located, it is suggested, between Ireland and Newfoundland, and passing north to the Sinus Borealis. By the late Triassic the rotation of Gondwana

Table 1 Average poles for the major continental blocks

<i>T</i>	<i>W</i>	<i>N(R)k</i>	$\lambda, \phi$	$\alpha_{95}$
North American poles ( $^{\circ}$ N, $^{\circ}$ E)				
20	20	10(09.95)175	88,171	4
30	20	10(09.89)82	86,152	5
40	20	07(06.91)64	85,167	8
50	40	12(11.76)45	78,173	6
60	40	11(10.81)51	73,186	6
70	40	11(10.87)79	70,187	5
80	40	09(08.91)85	69,192	6
90	40	08(07.93)95	69,194	6
100	40	05(04.99)458	69,190	4
110	40	04(03.99)503	67,186	4
120	40	05(04.92)52	68,179	10
130	40	06(05.89)44	67,169	10
140	40	07(06.75)24	72,152	13
150	40	08(07.77)31	72,147	10
160	40	08(07.83)40	76,131	9
170	40	07(06.87)45	76,114	9
180	40	09(08.80)41	72,106	13
190	40	07(06.91)64	70,094	8
200	40	07(06.96)134	64,093	5
210	40	11(10.84)61	59,100	6
220	40	07(06.92)79	55,103	7
230	40	08(07.81)37	53,108	9
240	40	09(08.84)50	49,114	7
250	40	06(05.94)82	43,120	7
260	40	09(08.79)38	42,115	9
270	40	10(09.78)40	43,117	8
280	40	08(07.77)31	39,121	10
290	40	11(10.72)36	38,121	8
300	40	10(09.90)90	38,126	5
310	40	10(09.91)95	37,124	5
320	40	11(10.86)72	36,126	5
330	40	07(06.93)85	36,129	7
340	40	06(05.93)73	33,126	8
350	40	05(04.92)50	35,128	10
Poles for northern Eurasia ( $^{\circ}$ N, $^{\circ}$ E)				
20	30	51(49.90)31	85,198	4
30	30	30(29.87)26	81,190	5
40	40	31(30.08)33	78,180	5
50	40	29(28.25)37	76,174	4
60	40	28(27.28)37	75,175	5
70	40	24(23.37)40	75,170	5
80	40	16(15.58)27	71,166	6
90	40	16(15.57)30	69,164	6
100	40	17(16.59)37	71,157	6
110	40	15(14.75)57	70,159	5
120	40	15(14.70)46	70,153	6
130	40	15(14.48)27	74,166	7
140	40	12(11.59)27	73,170	8
150	40	13(12.49)24	76,162	9
160	40	13(12.48)23	76,163	9
170	40	12(11.65)31	71,146	8
180	40	12(11.55)25	65,139	9
190	40	10(09.72)33	54,140	9
200	40	25(24.14)28	49,147	6
210	40	41(39.91)37	49,149	4
220	40	51(49.56)35	48,153	3
230	40	50(48.60)35	48,153	3
240	40	43(42.11)47	47,157	3
250	40	27(27.46)49	45,163	4
260	40	17(16.79)77	43,165	4
270	40	21(20.69)65	42,168	4
280	40	16(15.65)43	40,166	6
290	40	20(19.48)36	37,167	5
300	40	20(19.33)29	36,166	6
310	40	17(16.34)24	36,165	7
320	40	20(18.99)19	36,162	8
330	40	14(13.29)18	38,157	10
340	40	14(13.20)16	35,158	10
350	40	12(11.31)16	29,156	11
375	40	08(07.93)95	30,155	6
Poles from Gondwana ( $^{\circ}$ S, $^{\circ}$ E)				
160	30	08(07.89)63	60,076	7
170	30	12(11.80)53	63,076	6
180	30	12(11.84)70	64,077	5
190	30	09(08.86)57	67,074	7
200	30	07(06.92)80	67,076	7
210	30	10(09.84)56	68,072	6
220	30	11(10.85)69	58,072	6
230	40	12(11.56)25	56,072	6
240	40	08(07.86)51	51,077	8
250	40	05(04.90)40	41,076	12
260	40	05(04.96)98	36,068	8
270	40	05(04.96)103	37,067	8
280	40	06(05.96)118	38,068	6
290	40	05(04.99)406	40,065	4

*T* is geological time in Myr. *W* is the width of the averaging interval in Myr. *N* is the number of values used, *R* the resultant and *k* the precision<sup>48</sup>.  $\lambda, \phi$  are the latitude and longitude of the mean pole.  $\alpha_{95}$  is the 95% error. The data used are the starred entries in the Ottawa catalogues, and omitting results from deformed beds in foldbelts; for example, the selection for North America is essentially the same as that of ref. 50. Results from Speckled Sandstone of India<sup>51</sup> are also omitted. A list of the data used may be obtained from the author. The values for Gondwana have been rotated relative to Africa using the reconstruction of ref. 52.

relative to Laurasia stops, and the motion along *XM* reverses (Fig. 5*h*). North America is now locked to Gondwana, and both rotated anticlockwise relative to Europe, opening the western Tethys and forming Pangaea *A*. Mexico is assumed to move NW relative to North America<sup>33</sup>. During the transformation from Pangaea *B* to *A* it is as if Gondwana forces North America northwards out of its path, and then, carrying North America along with it, opens Tethys. The total relative motion along the Hercynian super-foldbelt is about 3,500 km. Movement is completed by the late Triassic, but its start is poorly determined. Some shear may have begun in the early Permian, and the time required may have been as long as 75 Myr (average relative velocity 5 cm yr<sup>-1</sup>). The northward movement of North America relative to Europe is at least 1,000 km, although the errors are such that the movement may be as much as 2,000 km (ref. 19). The succeeding southward movement of North America relative to Europe is of the same magnitude. The motion along *XM* is unlikely to be pure shear but also partly extensional, and one of the consequences might be the development of grabens, which, in the North Sea, contain oil. The formation of the early Triassic Siberian Traps, the dextral rotation of Novaya Zemlya, and the opening of the Kara Sea<sup>25</sup>, may also be caused by it. During the late Triassic, the Cordilleran super-foldbelt apparently develops as a continent-ocean interaction, and extends over 10,000 km from New Zealand to Alaska.

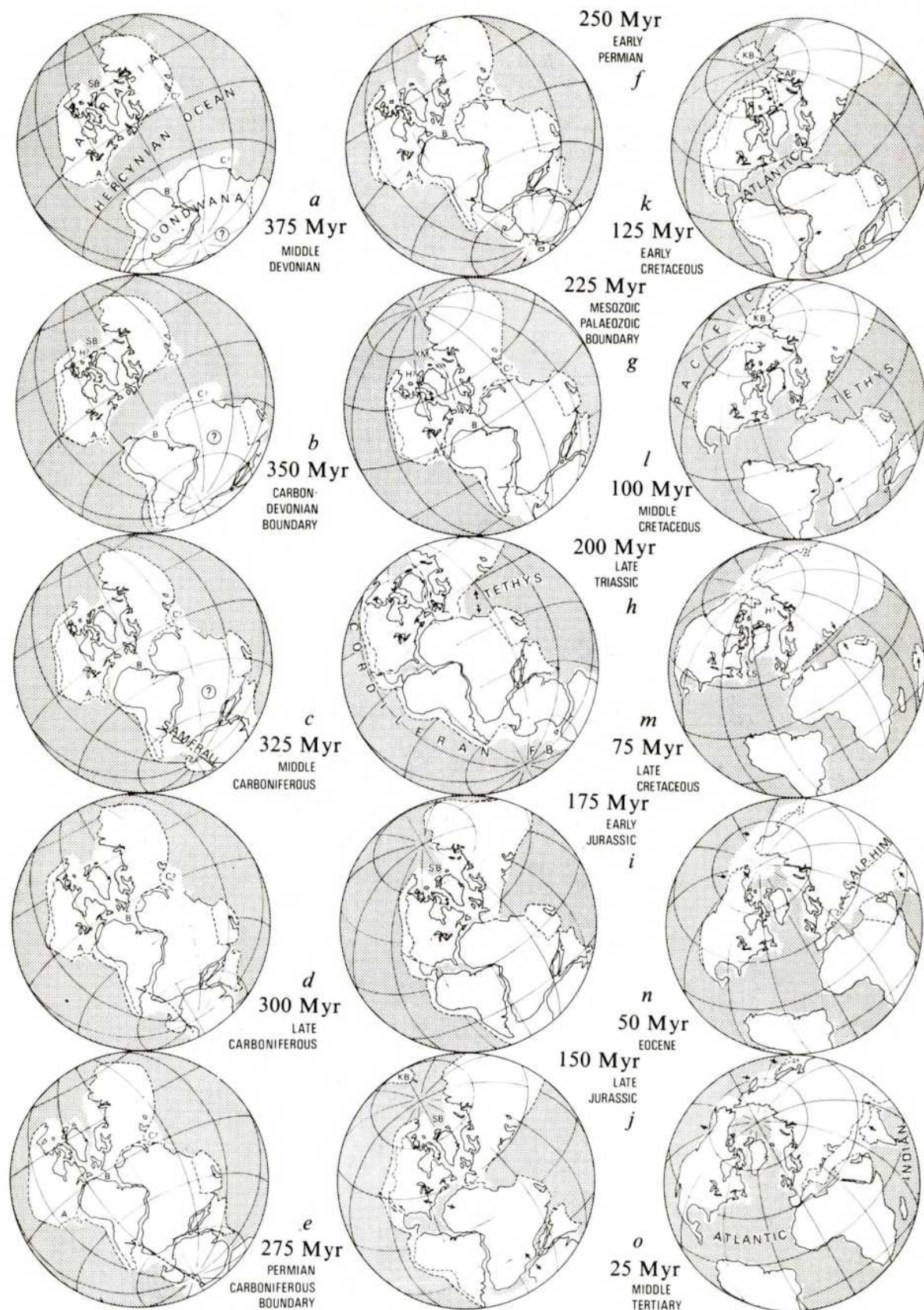
By the late Triassic or early Jurassic Wegener's Pangaea (Pangaea *A*) is achieved (Fig. 5*i*). By the mid-late Jurassic the North Atlantic begins to open, Mexico is able to achieve its present position, and a rift appears between eastern and western Gondwana. In the early Cretaceous, Sinus Borealis closes (Fig. 5*k*), and Spain begins to rotate anticlockwise. Rifting is initiated in the South Atlantic. In the Middle Cretaceous, Tethys is open to the Atlantic (Fig. 5*l*). The Kolyma Block, and surrounding Mesozoic foldbelts, could have been welded onto Eurasia<sup>27,34</sup> at this time. The region around the north pole is entirely covered with continental crust. During the late Cretaceous the Labrador Sea (*LS*) opens (Fig. 5*m*), Africa moves eastward relative to Europe, and Tethys closes to the west.

At the beginning of the Tertiary, NE Eurasia moves away from North America, allowing northern Alaska and parts of NE Siberia to rotate anticlockwise and open the Canada Basin (Fig. 5*n*); the Canada Basin could therefore be a back-arc basin formed by the migration of a thin sliver of continental crust over the NW Pacific subduction zone. India moves northward, and the Alpine-Himalayan super-foldbelt forms. By the mid-Tertiary, Alaska completes its rotation (Fig. 5*o*), the Atlantic rift system is fully developed, and the Alpine-Himalayan super-foldbelt is established in its present form.

## Discussion

The maps are presented as an explanation of the palaeomagnetic record. They have been constructed by adhering to the AGD assumption, within the statistical errors, that are within the limits for non-dipolar fields as they are presently known<sup>6</sup>. The maps indicate that Wegener's Pangaea lasted only a few tens of Myr, and that in the Permian and Mesozoic major continental blocks of North America, Eurasia and Gondwana were in more-or-less continuous motion relative to one another. Other workers, however, have taken a less mobilistic view, and their maps differ in two important respects. Firstly their maps<sup>49</sup> show the Arctic to be a static region throughout the Mesozoic, the bordering continents of North America and Eurasia being fixed relative to one another. But geologically the Arctic is far from static. There are foldbelts, rifts, subsiding basins and thick igneous sequences, that in other contexts would be taken as evidence of plate motions. Secondly, in most contemporary maps Wegener's Pangaea is regarded as inviolate in the Permian and Triassic, and the palaeomagnetic discrepancies of Fig. 4 are attributed to the presence of large non-dipole components in the geomagnetic field<sup>28,35,36</sup>, so that the palaeomagnetic pole





**Fig. 5** Continental drift since the Devonian. The latitudes of the major continents have been fixed by placing the palaeomagnetic poles (within their 95% error circles) at the poles of the projection using Geuer's Terrascope<sup>17</sup>. This rule is relaxed for *g* and *h* because it is not yet possible to provide satisfactory maps for the *B* to *A* transformation; the necessary details are unlikely to be revealed until a shorter averaging interval can be used, perhaps 20 Myr. *A*, *B* and *C* signify possible positions for micro-continents between Laurasia and Gondwana (see text); *AP* possible zone of subduction during closure of Sinus Borealis *SB*; *H*? possible position of the Chukotsk peninsula relative to Alaska; *KB* Kolyma Block; *LS* Labrador Sea; *XM* suggested location of Triassic shear zone between Europe and North America. The question-marks in Africa in *a*, *b* and *c* draw attention to the possible error in latitude determinations for Gondwana for the Devonian and Early Carboniferous, that arises from the uncertainty in the Devonian and Early Carboniferous pole path for Gondwana (Fig. 3). The width, indeed the existence of the 'Hercynian Ocean', is uncertain. This figure is an attempt to map the movements of the major continent blocks relative to one another and to the pole. No attempt is made to map the positions of minor blocks, and the boundaries of some of the major blocks (for example, North-East Gondwana, and South-East Asia) are highly speculative.

determinations, even when derived by averaging results from many rock units distributed over a large continental block, would be in error by as much as 20°. There is, however, excellent agreement between the palaeomagnetically determined latitudes and plate tectonic reconstructions for the Lower Jurassic onwards (Fig. 5*i* to *o*), so that if such large non-dipole components were present, they seem to have disappeared at the beginning of the Jurassic. There is no other evidence for such a change. Although the hypotheses of an inviolate Pangaea and large non-dipole fields in the late Palaeozoic and Mesozoic are still tenable, it is argued here that the alternative tectonic explanations of Fig. 5 are more reasonable; it would be a coincidence if the non-dipole fields were of exactly the form to produce the apparent latitude differences between North America and Eurasia at just the right time to allow the transformation from Pangaea *B* to *A* to occur, followed by the creation of Tethys when required by the geological evidence.

The concept of a mobile Pangaea provides a framework within which to discuss geological events. The Hercynian–Appalachian super-foldbelt could have developed as a result of the collision of Laurasia and Gondwana<sup>30</sup>. It is cut by large right-lateral wrench faults<sup>37,38</sup> and it has been suggested<sup>37</sup> that they were caused by the relative right-lateral displacement of Laurasia with respect to Gondwana; the displacement is estimated to be 600–1,000 km, and to have occurred between 290 and 250 Myr ago. It post-dates the main Hercynian Orogeny. The sense of displacement is the same, but it is three times less, and apparently began about 40 Myr earlier, than that of Fig. 5. Therefore there could be two diastrophic phases, the smaller earlier displacement of ref. 37 being followed by the larger displacement described here. The two phases could have overlapped in time. The earlier displacements at first met much resistance, and hence were slow and small, and were responsible for most of the observable geological strains. Once a zone of weakness was established, rapid displacements occurred, accompanied by less stress, and fewer observable geological effects. The location of the zone of weakness is not known, but it may have been between Spain and Morocco, and the Brevard Zone of the southern Appalachians is also a possible site. Alternatively, the dating of either the palaeomagnetic results, or the movements along the wrench faults, may be in error. Such large systematic errors are possible, but unlikely. Although the maps of Fig. 5 provide the framework for the discussion of the western part of the Hercynian super-foldbelt between Laurasia and Gondwana, they do not do so for the eastern part situated in central and eastern Asia. It is perhaps worthy of note that palaeomagnetic results from Malaya<sup>32</sup> and China<sup>1</sup> indicate that these blocks were in latitudes of 10 to 20°N during the Permian, and therefore they could have been situated in the eastern embayment between Africa and Asia (Fig. 5*e*). They could have formed part of one or more blocks that interacted with the southern edge of the Siberian craton to form the eastern part of the Hercynian super-foldbelt.

Pangaea *B* existed from the middle of the Carboniferous to the end of the Palaeozoic, about 80 or 90 Myr. It presumably indicates a special type of mantle convection at that time. It corresponds in time to the late Palaeozoic reversed interval (the Kiaman of ref. 39) when changes in the polarity of the geomagnetic field were very infrequent. The significance of this correlation can only be guessed. The onset of the *B* to *A* transformation coincides, within the accuracy of the age estimates, with the onset of frequent reversals at the end of the Permian. This first-order change in the geomagnetic field was probably a response to topographic or temperature changes at the core–mantle interface, and hence to changes in the lower mantle<sup>40,53</sup>. The *B* to *A* transformation differs from other known Phanerozoic drift episodes in that it does not seem to involve the creation of spreading ridges and new oceans between the major continental blocks. The absence of known spreading ridges is a possible cause of the worldwide regression at the end of the Permian. Exposure of the continental shelves, which house a high proportion of biological activity, could have

been the major environmental change responsible for the widespread extinctions at the end of the Palaeozoic<sup>41</sup>. The changes that occurred in mantle convection at the end of the Palaeozoic could have been responsible for both the sudden increase in reversal frequency, and the *B* to *A* transformation, and the latter, in its turn, caused the late Permian extinctions. The identification, made here, of a first-order episode of continental drift, that commenced at the very end of the Palaeozoic or earliest Triassic, may therefore be the major cause of the geological break between the Palaeozoic and Mesozoic. This drift episode, whose main feature was a dextral shear between the northern and southern continents about the palaeoequator, may also reflect a major change in mantle convection which produced what is perhaps the most important change in the geomagnetic field yet recorded.

Finally, the geological evidence indicates that during the late Palaeozoic there was apparently no ocean between Europe and Gondwana<sup>42</sup>, and Tethys did not open until the early Mesozoic (Fig. 5 *g* and *h* agree well with this evidence).

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*Note added in proof:* Recent palaeomagnetic studies in the Brooks Range have shown the anticlockwise rotation of northern Alaska<sup>55</sup>, thus confirming the indirect arguments given above.

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- Irving, E., Tanczyk, E. & Hastie, J. *Geomag. Ser. Earth Phys.* nos. 5, 6 & 10 (Branch, E.M.R. Ottawa, 1976).
- Khrumov, A. N. *Acad. Sci. USSR Sov. Geophys. Commun. WDC* 3, 1–43 (1975).
- Harland, W. B., Smith, A. G. & Wilcock, B. J. *geol. Soc., Lond.* 120, 250 (1964).
- Wilson, R. L. *Geophys. J. R. Astr. Soc.* 19, 417–437 (1970).
- Hide, R. *Science* 157, 55–56 (1967).
- Wilson, R. L. & McElhinny, M. W. *Geophys. J. R. astr. Soc.* 39, 570–586 (1974).
- Evans, M. E. *Nature* 262, 676–677 (1976).
- Irving, E. *Paleomagnetism* 1–399 (Wiley, New York, 1964).
- Crowell, J. C. & Frakes, L. A. *J. geol. Soc. Aust.* 17, 115–155 (1971).
- Phillips, J. D. & Forsyth, D. *Geol. Soc. Am. Bull.* 83, 1579–1600 (1972).
- Wegener, A. *Origin of Continents and Oceans* (Methuen, London, 1924).
- Le Pichon, X., Sibuet, J. C. & Francheteau, J. *Tectonophysics* (in the press).
- Bullard, E. C., Everett, J. E. & Smith, A. G. *Phil. Trans. R. Soc. A258*, 41–51 (1965).
- Van der Voo, R. & French, R. B. *Earth Sci. Rev.* 10, 99–119 (1974).
- Nairn, A. E. M. *Overseas Geol. Min. Res.* 9, 302–320 (1964).
- De Boer, J. *J. geophys. Res.* 70, 931–944 (1965).
- Van Hiltten, D. *Tectonophysics* 1, 3–71 (1964).
- Irving, E. *Systematics Ass. Spec. Publ.* no. 7, 59–76 (1967).
- Roy, J. L. *Earth planet. Sci. Lett.* 14, 103–114 (1972).
- Pitman, W. C. & Talwani, M. *Geol. Soc. Am. Bull.* 83, 619–640 (1972).
- McKenzie, D. & Sclater, J. G. *Geophys. J. R. astr. Soc.* 25, 437–528 (1971).
- Francheteau, J. *Implications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) 197–202 (Academic, New York 1973).
- Harland, W. B. *Arctic Geology* (ed. Pitcher, M. G.) *Am. Ass. Petrol. Geol.* 599–608 (1973).
- Tailleux, I. L. *Arctic Geology* (ed. Pitcher, M. G.) *Am. Ass. Petrol. Geol.* 526–535 (1973).
- Carey, S. W. *Continental Drift Symp.* 177–358 (University Tasmania, Hobart, 1958).
- Churkin, M. *Arctic Geology* (ed. Pitcher, M. G.) *Am. Ass. Petrol. Geol.* 485–499 (1973).
- Hamilton, W. *Geol. Soc. Am. Bull.* 81, 2553–2576 (1970).
- Smith, A. G., Briden, J. C. & Drewry, G. E. *Palaeont. Ass. Spec. Paper* 12, 1–42 (1973).
- Du Toit, A. L. *Our Wandering Continents* 1–366 (Oliver and Boyd, Edinburgh, 1937).
- McKerrow, W. S. & Zeigler, A. M. *Nature phys. Sci.* 240, 92–93 (1972).
- Zijdeveld, J. D. A. thesis, Univ. Utrecht (1975).
- McElhinny, M. W., Haile, N. S. & Crawford, A. R. *Nature* 252, 641–645 (1974).
- Van der Voo, F., Mauk, F. J. & French, R. B. *Geology* 2, 177–178 (1976).
- McElhinny, M. W. *Implications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) 77–85 (Academic, New York, 1973).
- Creer, K. M. *Applications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) 47–76 (Academic, New York, 1973).
- Briden, J. C., Smith, A. G. & Sallomy, J. T. *Geophys. J. R. astr. Soc.* 23, 101–117 (1971).
- Arthaud, F. & Matte, P. *Geol. Soc. Am. Bull.* (in the press); *Tectonophysics* 25, 139–171 (1975).
- Mattauer, M., Proust, F. & Tapponier, P. *Nature* 237, 160–162 (1972).
- Irving, E. & Parry, L. G. *Geophys. J. R. astr. Soc.* 7, 395–411 (1963).
- Irving, E. & Pullaiah, G. *Earth Sci. Rev.* 12, 35–64 (1976).
- Schopf, T. J. M. *J. Geol.* 82, 129–143 (1974).
- Argyriadis, I. *Bull. Soc. Geol. France* 17, 56–62 (1975).
- Wensink, H. *Progress in Geodynamics* 190–208 (R. Neith. Acad. Arts. Sci., 1975).
- Creer, K. M. *Earth Sci. Rev.* 6, 369–466 (1970).
- McElhinny, M. W. & Embleton, B. J. *Tectonophysics* 22, 1–29 (1974).
- Hailwood, E. A. *Earth planet. Sci. Lett.* 23, 376–386 (1974).
- Geuer, J. W. *Can. J. Earth Sci.* 10, 1164–1175 (1973).
- Fisher, R. A. *Proc. R. Soc. A217*, 295–305 (1953).
- Smith, A. G. & Briden, J. C. *Palaeocontinental Maps for the Mesozoic and Cenozoic* 63 (Cambridge University Press, Cambridge, 1977).
- Beck, M. E. *Am. J. Sci.* 276, 694–712 (1976).
- Wensink, H. *Tectonophysics* 26, 281–292 (1975).
- Smith, A. G. & Hallam, A. *Nature* 225, 139–144 (1970).
- Cox, A. *Rev. Geophys. Space Phys.* 13, 35–51 (1975).
- Westphal, M. *Nature* 267, 136–137 (1977).
- Newman, G. W., Mull, C. G. & Watkins, N. D. *Geol. Soc. Alaska, Abstracts, Anchorage meeting*, 16–19 (1977).



# X-ray bursts and neutron-star thermonuclear flashes

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*Some of the properties of thermonuclear flashes that should occur in the surface layers of accreting neutron stars are investigated. Such flashes may account for many of the observed properties of X-ray burst sources. Helium seems to be the most promising type of nuclear fuel for producing flashes that result in X-ray bursts.*

THE history of the discovery of X-ray burst sources has been described by Grindlay<sup>1</sup>, and their observed properties have been reviewed by Lewin<sup>2</sup> and Lewin and Joss<sup>3</sup>. These sources emit bursts of X-rays with risetimes of  $\lesssim 1$  s and decay time scales of  $\sim 3$ –100 s. If they are at a mean distance of  $\sim 10$  kpc, as suggested by their apparent concentration towards the direction of the galactic centre, then the X-ray energy emitted in each burst is typically  $\sim 10^{38}$ – $10^{40}$  erg. The maximum luminosity during the course of a burst is roughly equal to the Eddington limit for a  $1 M_{\odot}$  object ( $\sim 10^{38}$  erg s<sup>-1</sup>). The recurrence intervals between bursts are generally in the range  $\sim 10^4$ – $10^5$  s, except for the 'rapid burster' MXB1730–335 (ref. 4) which has recurrence intervals of  $\sim 10$ –100 s. Many, if not all, burst sources undergo extended inactive states lasting for weeks or longer, during which no bursts are seen. At least eight burst sources also seem to be associated with relatively steady X-ray sources, to within positional accuracies of  $\sim 0.1^{\circ}$ . The ratio,  $\alpha$ , of 'steady' X-ray luminosity to time-averaged burst luminosity is as large as  $\sim 250$  for the associated sources, but an upper limit as small as  $\alpha \lesssim 2$  has been placed on one burst source (MXB1730–335) with no identified steady counterpart.

A theoretical model of X-ray burst sources must account for these observations. Some models that have been suggested invoke mechanisms involving massive black holes<sup>5,6</sup> or instabilities in the accretion on to a compact object of roughly solar mass<sup>7–12</sup>. Here we discuss another type of model: the production of X-ray bursts by thermonuclear flashes in the freshly accreted matter near the surface of an accreting neutron star.

This model was first proposed by Maraschi and Cavaliere<sup>13</sup> and Woosley and Taam<sup>14</sup>. Even before the discovery of X-ray burst sources, the possibility of thermonuclear flashes on accreting neutron stars was considered by Hansen and Van Horn<sup>15</sup>. The present paper investigates further some of the physical processes relevant to such flashes in the context of the rapidly growing volume of observational information. This discussion is still highly preliminary, as realistic numerical calculations of such flashes have not yet been carried out; in particular, the characteristic timescale between flashes is unknown. We conclude that thermonuclear flashes may account for some, but not all, of the observed X-ray burst sources. Our discussion also considers why observational evidence has not yet been obtained for thermonuclear flashes in X-ray pulsars, which are also very likely to be accreting neutron stars. A discussion of some of the same points, including the physics of nuclear fusion processes in accreting neutron stars and the energetics of thermonuclear flashes, has been presented independently by Lamb and Lamb<sup>16</sup>.

## The physical picture

We consider a neutron star undergoing accretion of mass from a binary stellar companion. The infalling matter will be rich in hydrogen and/or helium. But, in the interior of the neutron star (density  $\rho \gtrsim 10^7$  g cm<sup>-3</sup> or depth  $h = R - r \gtrsim 10^2$  cm (see ref. 15),

where  $r$  is the distance from the centre of the neutron star and  $R$  is the neutron-star radius), nuclear reactions will proceed rapidly even at zero temperature and the nuclei will tend towards a state of nuclear statistical equilibrium. As the infalling matter accumulates, a given element of mass will pass through increasing density and must release the nuclear binding energy associated with the transmutation from its initial composition to a composition of iron-peak elements. If the accreted matter is pure hydrogen, then the energy released will be  $\sim 8 \times 10^{18}$  erg g<sup>-1</sup>.

If the neutron star is cold (internal temperature  $< 10^7$  K) and if the gravitational energy released by accretion is radiated away sufficiently rapidly, then the first reaction to proceed will be either pycnonuclear fusion or electron capture by protons at  $\rho \approx 10^7$  g cm<sup>-3</sup>, ultimately producing helium<sup>17</sup>. At somewhat higher densities, fusion of helium into heavier elements will proceed. These processes may occur quasi-statically<sup>18</sup>, especially if the temperature is very low<sup>17</sup>. If, however, either the accretion rate or the internal temperature of the neutron star is sufficiently high, then the fusion will be thermally unstable and should result in a thermonuclear flash; the thermonuclear runaway time scale for such a flash is usually  $\lesssim 1$  s (ref. 15). If the energy released in a flash can be transmitted to the neutron-star surface sufficiently rapidly and then lost as electromagnetic radiation, an X-ray burst will result.

We use here the models of Hansen and Van Horn<sup>15</sup> for neutron stars that are undergoing spherically symmetric accretion and steady-state nuclear burning, to estimate the conditions in the surface layers just before a flash. The actual conditions in the surface layers will be somewhat different in the absence of steady-state burning, but more accurate estimates cannot be made until time-dependent calculations of neutron-star envelopes undergoing nuclear burning are carried out.

## Energetics

Several useful constraints on thermonuclear-flash models can be derived from simple energetic considerations.

The gravitational energy per unit mass liberated by the accreting matter is given by  $E_a = (GM/R) = \eta_a c^2$ , where  $M$  is the mass of the neutron star, and  $\eta_a \sim 0.2$  is the ratio of released gravitational energy to rest-mass energy. (The value of  $\eta_a$  may be as small as  $\sim 10^{-3}$  or as large as  $\sim 0.4$ , depending upon the mass of the neutron star and the equation of state of matter at high densities.) This energy will be radiated away from the neutron-star surface with a time-averaged luminosity

$$L_a = \dot{m} E_a \approx 2 \times 10^{37} \left( \frac{\eta_a}{0.2} \right) \left( \frac{\dot{m}}{10^{17} \text{ g s}^{-1}} \right) \text{ erg s}^{-1} \quad (1)$$

where  $\dot{m}$  is the time-averaged mass accretion rate. In the case of the neutron stars that are the X-ray emitting components of binary X-ray pulsar systems, this luminosity is evidently emitted mostly as X radiation.

Nuclear fusion will also contribute to the total luminosity of an accreting neutron star<sup>18</sup>. The energy per unit mass released by nuclear fusion can be written as  $E_n = \eta_n c^2$ , where  $\eta_n$  is the energy efficiency of the reaction;  $\eta_n \approx 7 \times 10^{-3}$  for conventional stellar fusion of pure hydrogen (PP chains or CNO cycle),  $\eta_n \approx 1 \times 10^{-3}$  for helium-burning, and  $\eta_n \lesssim 1 \times 10^{-3}$  for the burning of heavier elements. As discussed below, full hydrogen-burning will not occur in a neutron-star thermonuclear flash, and we therefore

expect that in general  $\eta_n \lesssim 1 \times 10^{-3}$ . If X-ray bursts are caused by thermonuclear flashes in the accreted matter, then the ratio of the time-averaged accretion luminosity to the time-averaged luminosity in bursts is given by

$$\alpha_{\text{flash}} \simeq 2 \times 10^2 \left( \frac{\eta_n}{0.2} \right) \left( \frac{\eta_n}{10^{-3}} \right)^{-1} f^{-1} \xi^{-1} \quad (2)$$

where  $\eta_n$  is the efficiency factor appropriate to the nuclear reactions that participate in the flash,  $f$  is the fraction of the nuclear fuel that is consumed in flashes (rather than during the interflash periods), and  $\xi$  is the fraction of the flash energy that is transmitted to the neutron-star surface (rather than lost to the interior). The values of  $f$  and  $\xi$  are unknown, but we shall make the reasonable assumption that both quantities are of order unity. Thus, if the accretion on to the neutron star is steady on a timescale of hours or longer, then we expect that the time-averaged 'steady' X-ray flux from the neutron star will exceed the time-averaged burst flux by at least a factor of  $\sim 10^2$ .

It is also necessary that the accreted mass be sufficiently large to supply the thermonuclear energy of the flash. If a steady state has been established between the accretion of nuclear fuel by the neutron star and its consumption in flashes, then this constraint can be expressed as

$$\epsilon_B \simeq 9 \times 10^{38} \left( \frac{\eta_n}{10^{-3}} \right) \left( \frac{\dot{m}}{10^{17} \text{ g s}^{-1}} \right) \left( \frac{\tau}{10^4 \text{ s}} \right) f \xi \text{ erg} \quad (3)$$

where  $\epsilon_B$  is the average total energy emitted in each burst and  $\tau$  is the average time interval between bursts. (Only a fraction of the envelope above the burning shell need be consumed in a given flash, so that the total mass  $\delta m$  of the overlying surface layers may exceed  $\dot{m}\tau$  by a substantial factor.) If  $\dot{m}\tau$  is actually much less than  $10^{21} \text{ g}$ , then the flashes will be weak and will not produce observable X-ray bursts. The nuclear energy generation rate required to supply the flash energy  $\epsilon_B/\xi$  is given by

$$q_{\text{nuc}} \gtrsim 10^{17} \left( \frac{\epsilon_B}{10^{39} \text{ erg}} \right) \left( \frac{\delta m}{10^{21} \text{ g}} \right)^{-1} \left( \frac{\tau_B}{10 \text{ s}} \right)^{-1} f^{-1} \xi^{-1} \text{ erg g}^{-1} \text{ s}^{-1} \quad (4)$$

where  $\tau_B$  is the timescale of duration of the burst emission.

The energy of the flash is thermalised as it is transmitted to the neutron-star photosphere (see below), so that the characteristic photon energy of the burst will be

$$\begin{aligned} e_\gamma &\simeq 4k(\epsilon_B/4\pi\sigma R^2\tau_B)^{1/4} \\ &\simeq 6 \left( \frac{\eta_n}{10^{-3}} \right)^{1/4} \left( \frac{\dot{m}}{10^{17} \text{ g s}^{-1}} \right)^{1/4} \left( \frac{\tau}{10^4 \text{ s}} \right)^{1/4} \left( \frac{\tau_B}{10 \text{ s}} \right)^{-1/4} \times \\ &\quad \times \left( \frac{R}{10^6 \text{ cm}} \right)^{-1/2} f^{1/4} \xi^{1/4} \text{ keV} \end{aligned} \quad (5)$$

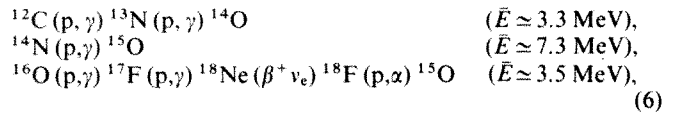
The indicated values of  $\epsilon_B$  and  $e_\gamma$  for the chosen plausible parameter values are consistent with the observed properties of X-ray burst sources.

## Nuclear reactions

We first consider hydrogen-burning as a possible source of nuclear energy. The density at the base of a steadily burning hydrogen-rich layer will be in the range  $10^4 \lesssim \rho_b \lesssim 10^5 \text{ g cm}^{-3}$  (ref. 15). (We shall use  $\rho_b$  and  $T_b$  to denote the density and temperature at the base of a layer of any specified nuclear fuel.) It seems probable that  $\rho_b$  will not lie very far outside this range even if the burning is unsteady.

For the PP chains, the reaction rate is too slow at any temperature to release the energy in a time  $\lesssim 10 \text{ s}$ , as would be required to account for the short timescales of the observed bursts. The CNO cycle is unable to operate fully, because the  $\beta$  decays of the intermediate nuclei ( $^{13}\text{N}$ ,  $^{15}\text{O}$ , and  $^{17}\text{F}$ ) require timescales of  $\sim 10^2$ – $10^3 \text{ s}$ . There are no known  $\beta$ -unstable excited states of any of these nuclei that could be thermally populated and thereby appreciably shorten the  $\beta$ -decay timescales, nor will the timescales be effectively reduced, at the expected densities, by captures of continuum electrons. Some of the  $\beta$ -unstable nuclei will undergo

additional proton captures during the flash, provided that the temperatures attained are sufficiently high ( $\gtrsim 10^9 \text{ K}$ ). The resultant reaction chains for initial  $^{12}\text{C}$ ,  $^{14}\text{N}$ , and  $^{16}\text{O}$  nuclei are as follows:



where  $\bar{E}$  is the average energy liberated per proton capture (excluding the average neutrino loss, if any). The  $\beta$ -decay half life of  $^{18}\text{Ne}$  is 1.5 s, so that this nucleus can decay sufficiently rapidly to continue through the reaction chain on a timescale less than  $\tau_B$ . Of course,  $^{13}\text{C}$ ,  $^{15}\text{N}$  and other heavier nuclei will participate in similar reaction chains, but their initial abundances are likely to be lower than those of  $^{12}\text{C}$ ,  $^{14}\text{N}$ , and  $^{16}\text{O}$ . (The daughter nuclei of  $^{14}\text{O}$  and  $^{15}\text{O}$  may be important if the same layer of matter participates in more than one flash, but this consideration does not substantially alter the expected values of  $\eta_n$ .)

An average of  $\sim 2$  protons will be consumed for each CNO nucleus, and the amount of energy liberated per CNO nucleus will be  $\sim 8 \text{ MeV}$ . Hence, if the duration of flash-burning is no greater than the observed duration of an X-ray burst, then the energy efficiency of the protonisation of CNO nuclei will be  $\eta_n \sim 9 \times 10^{-3} N_{\text{CNO}}$ , where  $N_{\text{CNO}}$  is the fractional abundance by number of CNO nuclei in the unburned matter. (We assume that  $N_{\text{CNO}} \lesssim 0.3$ .) This value of  $\eta_n$  will be increased slightly by proton captures on to other heavy nuclei and will also be increased by  $\alpha$ -captures, which should occur at the high temperatures attained in the flash. The value of  $\eta_n$  will thus depend on the temperatures achieved during the flash, as well as on whether the same layer of accreted matter participates in more than one flash. If the CNO abundance is near the cosmic value of  $N_{\text{CNO}} \sim 10^{-3}$ , however, then the energy efficiency of a hydrogen-burning flash should generally be quite low.

If, instead, the flash is caused by the fusion of helium or heavier elements, then the reaction rates will not be retarded by the effect of weak interactions and the reactions can proceed very swiftly ( $\ll 1 \text{ s}$ ) when sufficiently high temperatures are achieved. (The relevant nuclear reaction rates are not greatly affected by relativistic effects, electron degeneracy effects, or electron screening, so that conventional reaction-rate formulae may be used.) The energy efficiency of helium-burning is no lower than that of hydrogen-burning, unless the CNO abundance in a hydrogen-burning flash is extremely high ( $N_{\text{CNO}} \gtrsim 0.1$ ).

The value of  $\eta_n$  in a flash of helium or heavier elements may be increased by convective mixture. For example, such mixing could draw hydrogen into a helium-burning shell and result in proton-captures on to the heavier nuclei that are being synthesised.

Neutrino loss rates will be highest if the flash occurs in helium. The models of ref. 15 suggest that  $\rho_b \gtrsim 10^6 \text{ g cm}^{-3}$  at the base of the helium-rich layer; the released energy would be sufficient to raise the temperature to  $T_b \sim 2 \times 10^9 \text{ K}$  for  $\rho_b \sim 10^6 \text{ g cm}^{-3}$  (where a substantial fraction of the internal energy would be taken up by blackbody radiation) and to  $T_b \sim 10^{10} \text{ K}$  for  $\rho_b > 10^7 \text{ g cm}^{-3}$  (where the radiation energy density would be negligible). The neutrino loss rates (ref. 19; ref. 20 and references therein) are as high as  $\sim 10^{18} \text{ erg g}^{-1} \text{ s}^{-1}$  at  $\rho_b \sim 10^7 \text{ g cm}^{-3}$  and  $T_b \sim 10^{10} \text{ K}$ , but decrease rapidly with decreasing temperature and also decrease with increasing density. The effective helium-burning flash energy and the value of  $T_b$  may thus be slightly reduced in a narrow range of densities near  $\rho_b = 10^7 \text{ g cm}^{-3}$ , but will be unaffected at other densities. At the higher densities anticipated for a flash of heavier elements ( $\rho_b \gtrsim 10^9 \text{ g cm}^{-3}$  and  $T_b \sim 10^{10} \text{ K}$ ; see ref. 14), the neutrino loss rates are negligible. They will also be negligible in a hydrogen-burning flash, since  $T_b$  will then be no greater than  $\sim 10^9 \text{ K}$  unless  $N_{\text{CNO}}$  is extremely large ( $\gtrsim 0.1$ ).

## Heat transport

If a thermonuclear flash is to produce an observable X-ray burst, then the surface layers of the neutron star must be sufficiently thick

to provide the nuclear energy of the burst. One must, therefore, also consider whether the timescale for transport of the energy of the flash to the neutron-star surface will be  $\lesssim 1$  s, as required to account for the observed rapid risetimes of the X-ray bursts.

Conduction of heat by electrons is probably unimportant even if the electron degeneracy of the surface layers is high before the flash, because the degeneracy will generally be lifted and the conductivity will become small in each layer of matter before most of the heating of the layer by the flash has occurred. Convection should be important in the development of the flash, and an estimate based on the mixing-length theory indicates that the timescale for the initial transport of heat through the convectively unstable region will not greatly exceed the sound travel time ( $\ll 1$  s). In many possible circumstances, however, convection will not transport the energy of the flash all the way to the neutron-star photosphere. We demonstrate the validity of this statement as follows.

Consider first a hydrogen-burning flash. If all of the energy is released rapidly (in a time  $\ll 1$  s), and the CNO abundance is enhanced (say,  $N_{\text{CNO}} \sim 2 \times 10^{-2}$ ), then  $\sim 4$  MeV is released for each proton consumed, and a maximum temperature of  $T_b \sim 10^9$  K may be achieved at the base of the burning shell. This result is self-consistent, since the proton-capture reactions will, in fact, proceed very rapidly at  $T_b \sim 10^9$  K. Some burning of helium and heavier elements should also occur, but the temperature will not be increased greatly over this value. At this temperature, electron degeneracy will be lifted and the flashed matter will behave as a mixture of a nearly ideal gas plus blackbody radiation. We distinguish between two cases. (1) If  $\rho_b > 10^4$  g cm $^{-3}$ , then the internal energy of the gas dominates that of the radiation and

$$\beta \equiv \frac{\text{gas pressure}}{\text{total pressure}} \gtrsim 0.9$$

Hence, as a convective element rises and cools nearly adiabatically, its temperature,  $T_{\text{conv}}$ , will depend on its density,  $\rho_{\text{conv}}$ , as

$$T_{\text{conv}} \propto \rho_{\text{conv}}^{2/3} \quad (7)$$

(2) If, instead,  $\rho_b < 10^3$  g cm $^{-3}$ , then the radiation energy density becomes significant, so that  $\beta \lesssim 0.3$ ,  $T_b$  is somewhat reduced, and

$$T_{\text{conv}} \propto \rho_{\text{conv}}^{1/3} \quad (8)$$

For the greater energy per unit mass that might be released in a flash of helium or heavier elements, degeneracy is again generally lifted; for  $\rho_b > 10^8$  g cm $^{-3}$ , one obtains  $\beta \gtrsim 0.9$  and  $T_b \sim 10^{10}$  K, while for  $\rho_b < 10^7$  g cm $^{-3}$ , one obtains  $\beta \lesssim 0.3$  and somewhat smaller values of  $T_b$ .

In all cases where  $\beta \gtrsim 0.9$ , relation (7) implies that the temperature of a convective element will have fallen to  $T_p \lesssim 10^6$  K due to its expansion by the time it has reached the photosphere. This may well be less than the ambient photospheric temperature  $T_e$ , in which case the buoyancy of a convective element will vanish at a deeper level of the surface layers and no convective heat flux will reach the photosphere. Even if  $T_p$  is in excess of  $T_e$ , it is smaller by a factor of  $\gtrsim 10$  than the photospheric temperature required to produce the X-radiation of a burst (see equation (5)). On the other hand, if  $\beta \lesssim 0.3$ , then relation (8) implies that  $T_p \gtrsim 10^7$  K, so that convection can transport heat to the photosphere with adequate efficiency to produce an X-ray burst. But, for a flash of elements heavier than helium,  $\beta$  should always be close to unity and convection should, therefore, never be able to transport heat all the way to the photosphere (compare ref. 14).

The remaining heat transport mechanism of significance is radiative diffusion. From the equation of radiative transfer in local thermodynamic equilibrium, we find that under the conditions of a flash the radiative luminosity,  $L_{\text{rad}}$ , exceeds the Eddington limit  $L_{\text{crit}}$ . This implies that the heat transport will be dominated by convection up to a level where the temperature gradient is sufficiently small to allow  $L_{\text{rad}} \simeq (1 - \beta)L_{\text{crit}}$  (ref. 21). If  $\beta \lesssim 0.3$  at

the burning shell and convection therefore penetrates to the photosphere, the radiative luminosity escaping from the photosphere should still be  $\sim L_{\text{crit}}$ . For the outer portion of the surface layers, we may thus take

$$L_{\text{rad}} \sim (1 - \bar{\beta})L_{\text{crit}} \\ \simeq 1 \times 10^{38} (1 - \bar{\beta}) \left( \frac{M}{M_{\odot}} \right) \left( \frac{\kappa}{0.4 \text{ cm}^2 \text{ g}^{-1}} \right)^{-1} \text{ erg s}^{-1} \quad (9)$$

where  $\bar{\beta}$  is the typical value of  $\beta$  and  $\kappa$  is the typical radiative opacity in the outer surface layers (expressed in units of the Thomson scattering opacity for pure hydrogen).

Let  $\rho_r$  be the density at the base of the radiative layer when the convective motions first penetrate to that layer. Using equation (7) and the equations of hydrostatic equilibrium and radiative transfer, and assuming that  $L_{\text{rad}}$  and  $\kappa$  vary slowly through the outer surface layers before the flash, we can estimate  $\rho_r$  for cases where  $\beta \gtrsim 0.9$  at the burning shell:

$$\rho_r \sim 1 \times 10^5 \left( \frac{\rho_b}{10^8 \text{ g cm}^{-3}} \right)^2 \left( \frac{T_b}{10^{10} \text{ K}} \right)^{-3} \left( \frac{T_o}{10^7 \text{ K}} \right)^3 \text{ g cm}^{-3} \quad (10)$$

Here,  $T_o$  is the temperature before the flash at the level where  $\rho = 10^2$  g cm $^{-3}$ . The indicated values of  $\rho_b$  and  $T_b$  are plausible for a helium-burning flash<sup>15</sup>, and we expect that  $T_o \sim 10^7$  K in a neutron star undergoing a moderate rate of accretion. Using equation (10) and the equation of hydrostatic equilibrium, we further find that the timescale for the initial diffusion of radiation from the base of the radiative layer to the photosphere is

$$\tau_r \lesssim \kappa \rho_r h_r^2 / c \\ \sim 0.1 \left( \frac{\kappa}{0.4 \text{ cm}^2 \text{ g}^{-1}} \right) \left( \frac{\rho_b}{10^8 \text{ g cm}^{-3}} \right)^{10/3} \left( \frac{T_b}{10^{10} \text{ K}} \right)^{-5} \left( \frac{T_o}{10^7 \text{ K}} \right)^7 \\ \times \left( \frac{M}{M_{\odot}} \right)^{-2} \left( \frac{R}{10^6 \text{ cm}} \right)^4 \mu_r^{-2} \text{ s} \quad (11)$$

Here  $h_r$  is the depth of the initially radiative layer and  $\mu_r$  is the typical mean molecular weight in the layer.

These estimates of  $L_{\text{rad}}$  and  $\tau_r$  are crude, but they indicate that convection and/or radiative diffusion should transport sufficient heat from a helium-burning flash to the neutron-star photosphere to produce an X-ray burst and that the initial risetime of the burst should be sufficiently short to agree with the observations. From equation (3), we also find that the indicated value of  $L_{\text{rad}}$  is just sufficient to give a timescale for burst emission of  $\tau_B \sim \epsilon_B / L_{\text{rad}} \sim 10$  s. The luminosity and diffusion timescale for a hydrogen-burning flash are also capable of accounting for the properties of an X-ray burst, but  $\epsilon_B$  and  $\tau_B$  will be too small and  $\alpha_{\text{flash}}$  will be too large unless the CNO abundance is considerably enhanced over its cosmic value. A thermonuclear flash of carbon or heavier elements at  $\rho_b \gtrsim 10^9$  g cm $^{-3}$ ,  $T_b \sim 10^{10}$  K, and depth  $h_b \sim 10^4$  cm (ref. 14) can reproduce the emitted energies of X-ray bursts, but may not be able to produce sufficiently short burst risetimes.

Convection will carry fresh fuel into the burning shell to feed the flash. The total amount of available fuel may therefore exceed the mass of the initial burning shell by a large factor. On the other hand, the burning will tend to be terminated by the expansion and cooling of the surface layers following the initial flash. In the absence of detailed numerical calculations, one cannot determine what the duration of the flash will be. It is plausible, however, that the expansion of the surface layers and subsequent termination of the flash will occur on a thermal timescale for the surface layers:

$$\tau_{\text{th}} \sim 10 \left( \frac{2 - \beta_s}{\beta_s} \right) \left( \frac{T_s}{10^{10} \text{ K}} \right) \left( \frac{\delta m}{10^{21} \text{ g}} \right) \left( \frac{L_{\text{rad}}}{10^{38} \text{ erg s}^{-1}} \right)^{-1} \mu_s^{-1} \text{ s} \quad (12)$$

where  $\beta_s$ ,  $T_s$ , and  $\mu_s$  are the typical values of  $\beta$ , temperature, and

mean molecular weight in the surface layers following the flash. (Note that  $\tau_{th}$  must be comparable to  $\tau_B$ , since the internal energy of the surface layers is dominated by the energy released in the flash.) If all of the available nuclear fuel is consumed in a time less than  $\tau_{th}$ , then the flash will instead terminate on a nuclear-exhaustion timescale:

$$\tau_{nuc} \sim 10 \left( \frac{\eta_n}{10^{-3}} \right) \left( \frac{\delta m}{10^{21} \text{ g}} \right) \left( \frac{L_{nuc}}{10^{38} \text{ erg s}^{-1}} \right)^{-1} f \text{ s} \quad (13)$$

where  $L_{nuc}$  is the integrated nuclear energy generation rate throughout the surface layers; one expects  $L_{nuc} > L_{rad}$  during the flash. Either (or both) of these timescales can be comparable to or shorter than 10 s, as desired if thermonuclear flashes are to account for the observed bursts.

The emergent radiation will be effectively thermalised. A  $\sim 10$  MeV photon produced in a  $(p, \gamma)$  reaction, for example, will be thermalised to  $\sim 10$  KeV after  $\sim 10^2$  scattering events. Hence, most photons generated at an optical depth greater than  $\sim 10$  will be thermalised by the time they reach the photosphere. The presence of convection should not inhibit the thermalisation; from the mixing-length theory, we estimate that a photon produced in the burning shell will generally undergo many scattering events before reaching the photosphere. Moreover, even if thermonuclear fusion spreads through most of the surface layers during the progress of the flash, a significant amount of fusion should not reach an optical depth as small as  $\sim 10$ . The transport of radiation through the accreting matter, however, may complicate the emergent spectrum, as seems to be the case for the binary X-ray pulsars (see ref. 22 and references therein).

## Conclusions

The surface layers of accreting neutron stars should undergo thermonuclear flashes that will produce unsteady radiation, and this radiation may have properties similar to those of the observed X-ray burst sources. Helium-burning flashes are the most promising type for producing X-ray bursts, because of the relatively high energy yield and because the timescale for the released heat to reach the neutron-star photosphere is shorter than for flashes that result from the burning of carbon and other heavier elements. Enhanced CNO abundances ( $N_{CNO} \gtrsim 10^{-2}$ ) are needed if hydrogen-burning flashes are to be sufficiently energetic.

The burning of heavier elements within an accreting neutron star is likely to be thermally unstable under a wide range of conditions<sup>14</sup>. The effects of such instabilities could be more difficult to observe than the X-ray bursts detected, since the radiation may well be emitted at characteristic photon energies considerably less than 10 KeV and on timescales considerably longer than 10 s.

For thermonuclear flashes under nearly all possible circumstances, the value of  $\alpha_{flash}$  will be  $\gtrsim 10$ ; the most plausible values of  $\alpha_{flash}$  are  $\gtrsim 10^2$ . (The smallest values of  $\alpha_{flash}$  will be attained for low-mass neutron stars, with small values of  $\eta_n$ , or for hydrogen-burning flashes involving highly enhanced CNO abundances, with large values of  $\eta_n$ .) Hence, a thermonuclear-flash model cannot account for MXB1730-335 ( $\alpha \lesssim 2$ ) unless one inserts an additional *ad hoc* complication into the model, such as highly anisotropic beaming of the steady emission so that it largely misses the Earth. But, the empirical difference between the behaviour of MXB1730-335 and other burst sources (refs 2, 3 and references therein) suggests that the fundamental nature of this source may be unique. The burst source MXB1659-29, which has recently been found to have  $\alpha < 25$  (W. Lewin, J. Hoffman, and J. Doty, unpublished SAS-3 data), may also pose a serious problem for thermonuclear-flash models.

Another difficulty with thermonuclear-flash models is their prediction of simple blackbody spectra for X-ray bursts, in contrast to the rather complex changes in spectrum that are displayed during the progress of some observed bursts<sup>2,3</sup>. To account for such behaviour, it is necessary to invoke time-dependent reprocessing of the burst radiation by matter above the

Alfvén surface of the neutron star. It is worth noting, however, that the time-averaged burst spectra more closely resemble a blackbody than a thin-bremsstrahlung or power-law spectrum<sup>2</sup>. Moreover, the instantaneous spectra of at least three burst sources seem to be well-represented by a blackbody with an effective radius of  $\sim 10$  km throughout the course of each burst<sup>2,3-25</sup>.

On the other hand, thermonuclear-flash models may provide a natural explanation for the quasi-periodic character of the recurrence intervals that have been observed in several burst sources<sup>2,3</sup>. If the accretion rate on to the neutron star is nearly constant on a timescale of days and each burst consumes about the same amount of fuel, then a nearly constant interval between bursts may result (since a specific envelope mass may be needed to precipitate a flash). The observed variations in the recurrence intervals on timescales of several days to weeks could result from variations in the accretion rate on such timescales, while the cessation of bursting in many sources for periods of months or longer may be caused by either (1) a sharp reduction in the accretion rate or (2) an increase in the accretion rate that changes the properties of the neutron-star surface layers so as to alter the character of the nuclear fusion processes.

It is somewhat puzzling that all accreting neutron stars do not display some evidence of thermonuclear flashes. There are 12 known X-ray pulsars, which are widely believed to be accreting neutron stars (see ref. 22 and references therein) but which have not been observed to burst. It is possible that the X-ray burst sources are intrinsically very similar to the X-ray pulsars, but with lower accretion rates or other differences in system parameters<sup>10</sup>. But, in all cases investigated by Hansen and Van Horn<sup>15</sup>, the nuclear burning shells were either thermally unstable or at most marginally stable. These models do show a slight trend toward greater stability of nuclear burning as the mass accretion rate increases, but instability was found even at accretion rates corresponding to steady X-ray luminosities of as much as  $\sim 10^4 L_\odot$ . A better understanding of these problems must await a detailed time-dependent numerical treatment of nuclear fusion processes in neutron-star surface layers.

As noted above, it is possible that more than one mechanism is responsible for the observed burst sources. In order to distinguish between models invoking massive black holes<sup>1,5,6</sup> and those which involve moderate-mass compact objects<sup>7-12</sup> that may be in binary systems, observations of X-ray pulsing, X-ray eclipses, or optical companion stars which exhibit binary characteristics (ellipsoidal light variations or orbital velocity curves) could prove decisive.

It is equally important and, in principle, more straightforward to distinguish between thermonuclear-flash models for burst sources and those models which involve instabilities in accretion on to a neutron star<sup>7-11</sup>. This could be accomplished by determining the distribution of values of  $\alpha$  for a large statistical sample of sources. If the value of  $\alpha$  for most sources were  $\gtrsim 10^2$ , strong support for thermonuclear-flash models would be provided. If, on the other hand, there exists a relatively continuous distribution of values of  $\alpha$  extending well below  $10^2$ , then one could reasonably conclude that nuclear flash-burning is not responsible for any of the burst sources.

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- Grindlay, J. E. *Comm. Astrophys. Space Phys.* **6**, 165 (1977).
- Lewin, W. H. G. *Mon. Not. R. Astr. Soc.* **179**, 43 (1977); *Ann. N.Y. Acad. Sci.* (in the press).
- Lewin, W. H. G. & Joss, P. C. *Nature* **270**, 211-216 (1977).
- Lewin, W. H. G. *et al. Astrophys. J. Lett.* **207**, 135 (1976).
- Grindlay, J. & Gursky, H. *Astrophys. J. Lett.* **205**, L131 (1976).
- Bahcall, J. N. & Ostriker, J. P. *Nature* **262**, 37 (1976).
- Svestka, J. *Astrophys. Space Sci.* **45**, 21 (1976).
- Henriksen, R. N. *Astrophys. J. Lett.* **210**, L19 (1976).
- Baan, W. A. *Astrophys. J.* **214**, 245 (1977).
- Joss, P. C. & Rappaport, S. *Nature* **265**, 222 (1977).
- Lamb, F. K., Fabian, A. C., Pringle, J. E. & Lamb, D. Q. *Astrophys. J.* (in the press).
- Wheeler, J. C. *Astrophys. J.* **214**, 560 (1977).
- Maraschi, L. & Cavaliere, A. *Highlights of Astronomy* **4** (in the press).
- Woosley, S. E. & Taam, R. E. *Nature* **263**, 101 (1976).
- Hansen, C. J. & Van Horn, H. M. *Astrophys. J.* **195**, 735 (1975).



16. Lamb, D. Q. & Lamb, F. K. *Astrophys. J.* (submitted).
17. Van Horn, H. M. & Hansen, C. J. *Astrophys. J.* **191**, 479 (1974).
18. Rosenbluth, M. N. et al. *Astrophys. J.* **184**, 907 (1973).
19. Beaudet, G., Petrosian, V. & Salpeter, E. E. *Astrophys. J.* **150**, 979 (1967).
20. Barkat, Z. A. *Rev. Astr. Astrophys.* **13**, 45 (1975).
21. Joss, P. C., Salpeter, E. E. & Ostriker, J. P. *Astrophys. J.* **181**, 429 (1973).

22. Rappaport, S. & Joss, P. C. *Nature* **266**, 123 (1977).
23. Swank, J. H. et al. *Astrophys. J. Lett.* **212**, L73 (1977).
24. Hoffman, J., Lewin, W. H. G. & Doty, J. *Mon. Not. R. astr. Soc.* **179**, 57P (1977).
25. Hoffman, J. A., Lewin, W. H. G., Doty, J., Jernigan, J. G. & Haney, M. *Astrophys. J. Lett.* (in the press).

# Ovalbumin gene is split in chicken DNA

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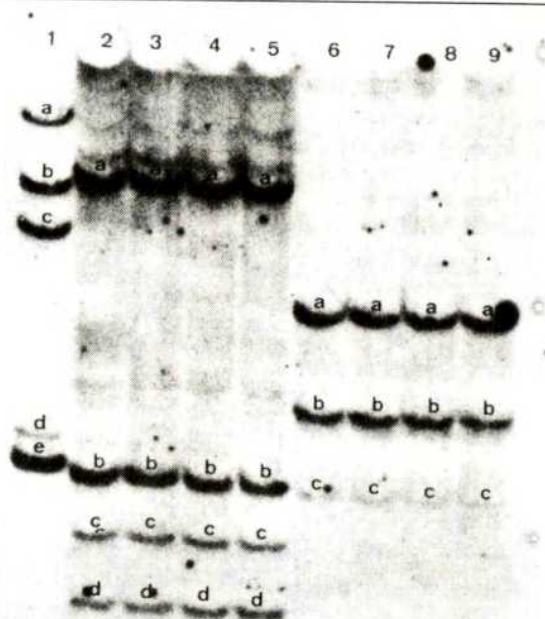
*The ovalbumin gene, is split in chicken DNA. Two interruptions in the sequences coding for ovalbumin mRNA have been detected, at least one of them lying in the protein coding sequence. The unexpected gene organisation is present both in oviduct cells highly specialised in ovalbumin synthesis and in erythrocytes.*

DNA SEQUENCES coding for the variable and constant regions of immunoglobulin molecules are widely separated in embryo cells but are much closer in differentiated lymphocytes<sup>1</sup>. This finding suggests that gene rearrangement could be one of the mechanisms involved in cell differentiation. Chicken oviduct is particularly useful for the study of such a possibility, since administration of oestrogen to immature chicks results in cytodifferentiation of tubular gland cells, which comprise up to 90% of the cells in the magnum portion of laying hen oviduct and which synthesise the major egg white proteins including ovalbumin, ovomucoid, conalbumin and lysozyme (see refs 2, 3). In particular, ovalbumin accounts for 50–65% of total protein synthesis in tubular gland cells, and the rate of transcription of its mRNA (which represents 40% of the total mRNA population) is under hormonal control<sup>3,4</sup>.

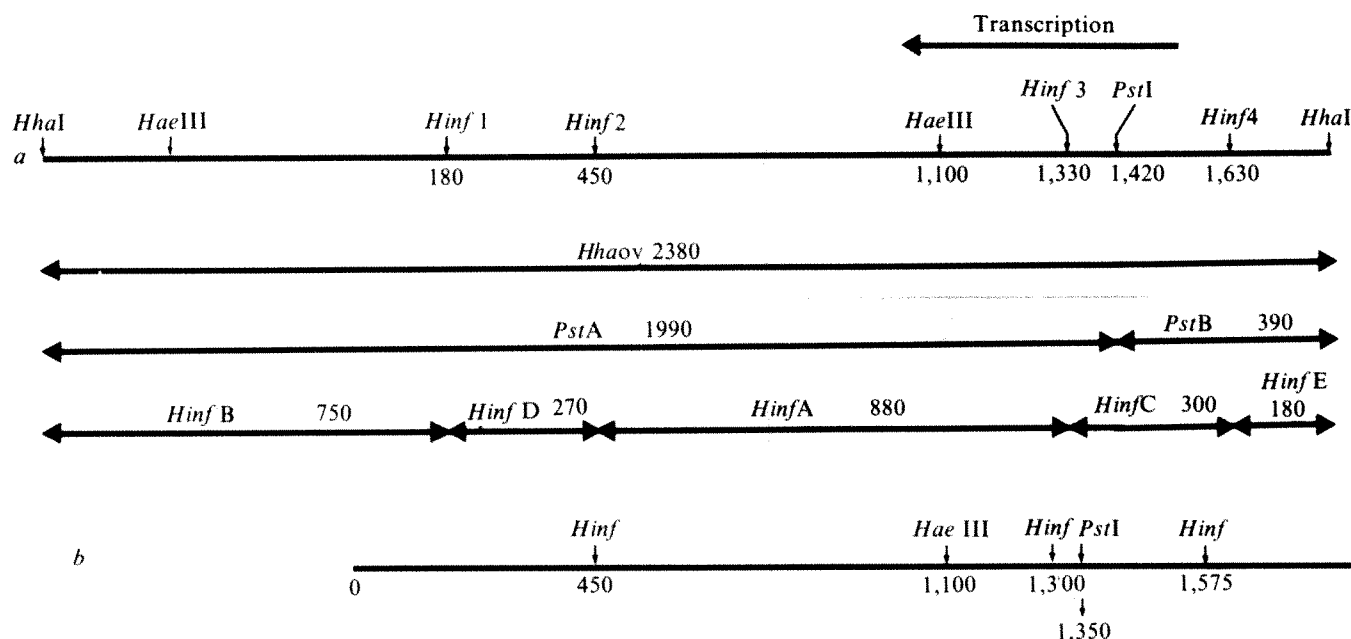
As reported for integrated viral genes<sup>5</sup>, it is possible to construct a physical map of restriction endonuclease sites within and around a single-copy gene. Briefly, this involves cleavage of the DNA with various restriction endonucleases, fractionation of the fragments by electrophoresis, their transfer to nitrocellulose sheets<sup>6</sup>, hybridisation to a specific probe labelled *in vitro* by nick-translation<sup>7</sup> to high specific activity and location of the gene fragments by autoradiography. In this study we have used the bacterial plasmid, pCR1ov2.1, which contains about 80% of the nucleotide sequences present in ovalbumin mRNA<sup>8</sup> as a hybridisation probe to investigate the possibility that the ovalbumin gene environment could be different in DNA prepared from laying hen oviduct or erythrocytes. Unexpectedly, we have found that the DNA sequences complementary to ovalbumin mRNA are split into several fragments in oviduct DNA, and that the same peculiar ovalbumin gene organisation is present in laying hen oviduct and erythrocyte DNA.

## Mapping chicken ovalbumin gene in oviduct and erythrocyte DNA

The *HhaI* fragment A (termed *Hhaov*) of the ovalbumin clone pCR1ov2.1 is about 2,400 base pairs long and contains a sequence of about 1,730 base pairs coding for ovalbumin mRNA<sup>8</sup>. After nick-translation with <sup>32</sup>P-labelled deoxyribonucleoside triphosphates<sup>7</sup>, this fragment serves as a filter hybridisation probe to detect DNA fragments containing the ovalbumin gene in restriction endonuclease digests of chicken DNA, using the blotting technique developed by Southern<sup>6</sup>.



**Fig. 1** Detection of ovalbumin gene sequences in chicken DNA cleaved with *EcoRI* or *HindIII*. DNA from the magnum portion of laying hen oviduct or from erythrocyte nuclei was purified as in refs 10 and 11, respectively. DNA samples were digested to completion with the restriction enzymes *EcoRI* or *HindIII* (gifts of Dr J. Sümegi). To monitor the extent of digestion, phage  $\lambda$  DNA was included in the incubations. Some digests were treated with proteinase K, phenol extracted, ethanol precipitated and redigested in the presence of fresh phage  $\lambda$  DNA to ensure complete cleavage. Samples of the digested DNA (10  $\mu$ g) were electrophoresed on 0.7% Agarose gels (17 cm long, 4 mm thick, lane width 8 mm) at 2 V  $\text{cm}^{-1}$ , transferred to nitrocellulose filters (Schleicher and Schell BA85) by a modification of the method of Southern<sup>6</sup>, and fixed to the filter by baking in a vacuum at 80 °C for 3 h. Before hybridisation, filters were soaked at 68 °C in 5  $\times$  SSC, 0.1 M sodium phosphate pH 7.0 containing 0.02% each of Ficoll, bovine serum albumin and polyvinylpyrrolidone<sup>12</sup>. Hybridisation to <sup>32</sup>P-labelled *Hhaov* fragment (0.1  $\mu$ g, 0.5–2  $\times 10^7$  c.p.m.) labelled by nick-translation<sup>7</sup> was carried out in the soaking solution for 16 h at 68 °C (ref. 5). The filters were washed first with the soaking solution containing 0.5% SDS, then extensively with 1  $\times$  SSC, 0.1 M sodium phosphate pH 8.4, containing 0.5% SDS. All washing was done at 68 °C. Autoradiography was for 7 d at room temperature with a Kodirex film (Kodak). *Hhaov* fragment was purified from an *HhaI* digest of pCR1ov2.1 DNA by sedimentation in a 5–20% sucrose gradient. Lane 1, Internal markers for molecular weight and hybridisation were prepared from pCR1ov2.1 DNA by cleavage with various restriction nucleases. Only fragments containing ovalbumin gene sequences are revealed after hybridisation. The molecular weight of the fragments was determined using adenovirus 2 DNA cleaved by *EcoRI* or *BamHI* as standard<sup>14</sup>. The enzymes generating the observed bands and the sizes of the fragments (in kilo base pairs) are: a, *HindIII*, 13.5; b, *SmaI*, 8.3; c, *HindIII/PstI*, 6.5 (contains the sequences present in *PstI*A fragment (see Fig. 2)); d, *HindIII/PstI*, 2.6 (contains sequences present in *PstI*B fragment (see Fig. 2)); e, *HhaI*, 2.4. Lanes 2–5, *EcoRI* digests of erythrocyte (lanes 2, 3) or oviduct (lanes 4, 5) DNA. Samples in lanes 3 and 5 were digested twice (see above). The sizes of the fragments hybridising to *Hhaov* are (in kilo base pairs): a, 9.5; b, 2.35; c, 1.8; d, 1.3. Lanes 6–9, *HindIII* digest of erythrocyte (lanes 6, 7) or oviduct (lanes 8, 9) DNA. Samples in lanes 7 and 9 were digested twice (see above). The sizes of the fragments hybridising to *Hhaov* are (in kilo base pairs): a, 4.9; b, 3.2; c, 2.3.



**Fig. 2** *a*, Physical map of restriction enzyme sites in the *Hhaov* fragment and orientation of the ovalbumin double-stranded cDNA insert (J.L.M., in preparation). Only the sites relevant to the present study are shown (for convenience, restriction endonuclease *HinfI* is termed *Hinf*). The position of the restriction endonuclease sites on the *Hhaov* fragment is given from the 3' end of the mRNA (excluding the poly (A) tail) assuming that the site *HinfI* corresponds to that predicted from the known sequence of ovalbumin mRNA around position 180 from its 3' end<sup>15</sup>. This assumption is supported by the demonstration that the *HinfB* fragment hybridises to mRNA (J.L.M., in preparation). Since the cloned ovalbumin ds cDNA sequence is about 1,730 nucleotides long<sup>8</sup>, all restriction sites from *HinfI* to *Hinf4* inclusive are located in a DNA sequence complementary to ovalbumin mRNA. Location and sizes of all fragments that have been used as hybridisation probes are also shown. *b*, Physical map of relevant restriction endonuclease sites of *in vitro* synthesised ovalbumin ds cDNA as established by Monahan *et al.*<sup>9</sup>. The position of the restriction enzyme sites is given from the 3' end of the mRNA. The two maps are in very good agreement when taking into account that different electrophoresis gel systems and molecular weight markers were used in the two studies.

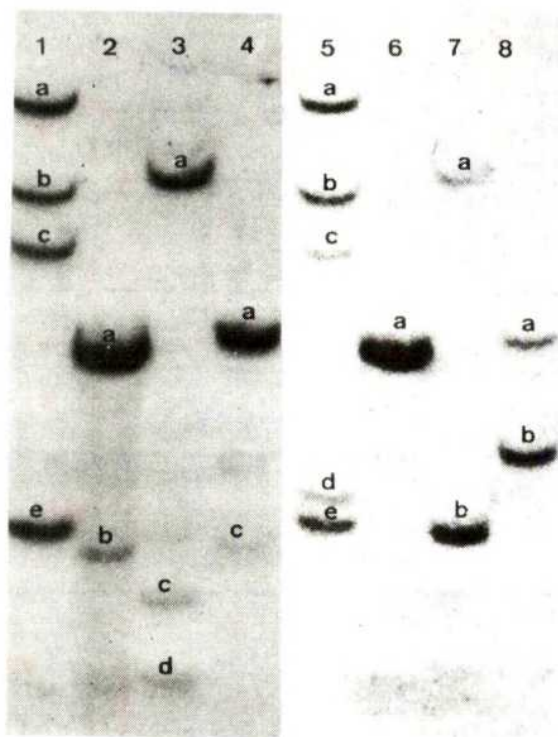
High molecular weight DNA was prepared from chicken oviduct and erythrocytes, digested with the restriction enzymes *EcoRI* or *HindIII*, electrophoresed on Agarose gels, denatured, and transferred to nitrocellulose filters<sup>6</sup>. After hybridisation with denatured <sup>32</sup>P-labelled *Hhaov* fragment and autoradiography, several bands were seen as shown in Fig. 1, suggesting that sequences complementary to ovalbumin mRNA are present in different DNA fragments. The autoradiography band pattern was identical for oviduct and erythrocyte DNA (Fig. 1) and also for 5-d-old chick embryo DNA (not shown). This multiplicity of hybridisation bands was unexpected, since there is no *EcoRI* or *HindIII* restriction enzyme site in the cloned ovalbumin cDNA sequences (Fig. 2). Absence of such sites in *in vitro* synthesised ovalbumin double-stranded cDNA (ds cDNA) was also recently reported by Monahan *et al.*<sup>9</sup>. The very good agreement between the restriction maps of the cloned and *in vitro* synthesised ds cDNAs (Fig. 2), together with our previous results<sup>8</sup>, exclude the possibility that the multiplicity of the bands can be attributed to DNA sequence rearrangements which might have occurred during the cloning and/or amplification of the ds cDNA. The following results exclude the possibility that the multiple hybridisation bands could be related to incomplete restriction enzyme digestion of the chicken DNA or to nonspecific hybridisation: (1) there was no change in the pattern when the DNA was digested twice, while the phage  $\lambda$  DNA internal marker was digested each time to completion (Fig. 1); (2) the hybrid bands were present even in much more stringent washing conditions (0.1 SSC, 0.5% sodium dodecylsulphate, 68 °C) and were as stable as the hybrids formed with the internal hybridisation markers (see legend to Fig. 1); (3) no hybridisation bands were observed when *EcoRI* DNA fragments of high molecular weight calf thymus DNA were used instead of *EcoRI* DNA fragments of high molecular weight calf thymus DNA were used instead of *EcoRI* fragments of chicken DNA (not shown); (4) the same bands were observed when the *Hhaov* probe was replaced by a probe from which plasmid sequences and GC tails adjacent to the ovalbumin sequences were removed by hy-

bridisation with ovalbumin mRNA followed by S1 nuclease treatment, as described in the legend to Fig. 4.

### Hybridisation to probes complementary to defined parts of ovalbumin mRNA

To investigate the significance of the multiple hybridisation bands we have prepared the <sup>32</sup>P-labelled fragments *PstA*, *PstB* and *HinfA* (see legend to Fig. 3). As illustrated in Fig. 2, the *PstA* probe contains most of the DNA sequences complementary to the first 1,420 nucleotides of the mRNA, starting from its 3' end. The *PstB* probe contains DNA sequences complementary to the 5' region of the mRNA from positions 1,420 to at least 1,630 from the 3' end of the mRNA. The *HinfA* probe contains only sequences complementary to the central part of the mRNA (Fig. 2). The hybridisation patterns of <sup>32</sup>P-labelled *PstA* and *PstB* probes to *EcoRI* or *HindIII* restriction fragments of oviduct DNA are shown in Fig. 3. The *PstA* probe hybridised with *EcoRI* fragments a, c and d, but not with fragment b (lane 3). The *HinfA* probe gave results very similar to those obtained with the *PstA* probe (not shown). On the other hand, the *PstB* probe hybridised strongly to the *EcoRI* fragment b, and only weakly to the *EcoRI* fragment a (lane 7). A similar pattern was found with *HindIII* fragments, since the *HindIII* fragment b did not hybridise with *PstA* (lane 4) or *HinfA* (not shown) probes, whereas it was responsible for the main band with the *PstB* probe (lane 8); this latter probe hybridised only weakly to *HindIII* fragment a. But, the *PstB* probe seemed to be contaminated with *PstA* sequences. Annealing of the *PstB* probe to the pCR10v2.1 fragments serving as internal markers showed indeed that it hybridised with both fragments c and d (Fig. 3, lane 5), which contain the *PstA* and the *PstB* DNA sequences respectively (J. L. Mandel, in preparation and legend to Fig. 1). On the other hand, the *PstA* probe hybridised only to the internal marker fragment c and not to the





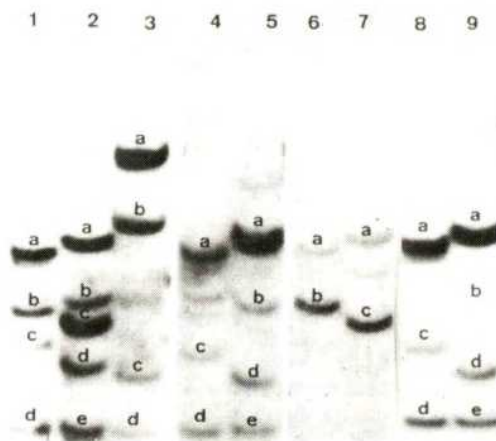
**Fig. 3** Detection of fragments containing sequences complementary to defined parts of ovalbumin mRNA in digests of chicken oviduct DNA with *EcoRI*, *HindIII* or *PstI*. DNA samples were prepared as described in Fig. 1, except that SV40 DNA was used to monitor the digestions with *PstI* (Biolabs). *PstA* and *PstB* probes were labelled by nick-translation of a *PstI* digest of *Hhaov*. After electrophoresis on a 2% agarose gel, the labelled *PstA* and *PstB* fragments were extracted from the gel<sup>16</sup>. Their specific activity was about  $1 \times 10^8$  c.p.m.  $\mu\text{g}^{-1}$ . Autoradiography was carried out with 'pre-flashed' Kodak RP Royal X-Omat film and intensifying screen at  $-70^\circ\text{C}$  for 7 d. All other procedures were as described in Fig. 1. Lanes 1-4, hybridisation to  $^{32}\text{P}$ -*PstA*. Lanes 5-8, hybridisation to *PstB*; lanes 1 and 5, internal markers (see Fig. 1). Lanes 2 and 6, *PstI* digest of chicken DNA—sizes of the fragments in kilo base pairs; a, 4.7; b, 2.2. Lanes 3 and 7, *EcoRI* digest of chicken DNA (see Fig. 1 for fragment sizes). Lanes 4 and 8, *HindIII* digest of chicken DNA (see Fig. 1 for fragment sizes).

marker fragment d (Fig. 3, lane 1). This indicates that the *PstA* probe did not contain sequences complementary to *PstB*, and that the hybridisation of the *PstB* probe to the marker fragment c was due to a contamination of that probe. This contamination could be due to some degradation of the *PstA* fragment during  $^{32}\text{P}$ -labelling, since the two probes were separated by gel electrophoresis after the nick translation reaction (see legend to Fig. 3). In any case, assuming that the weak hybridisation of the *PstB* probe to the *EcoRI* and *HindIII* fragments 'a' were due to such a contamination, our results with probes *PstA* and *PstB* suggested the possibility that, in the cellular DNA, some sequences complementary to the 5' moiety of the ovalbumin mRNA are physically separated from those complementary to the rest of the mRNA.

### Confirmation that the chicken ovalbumin gene is split

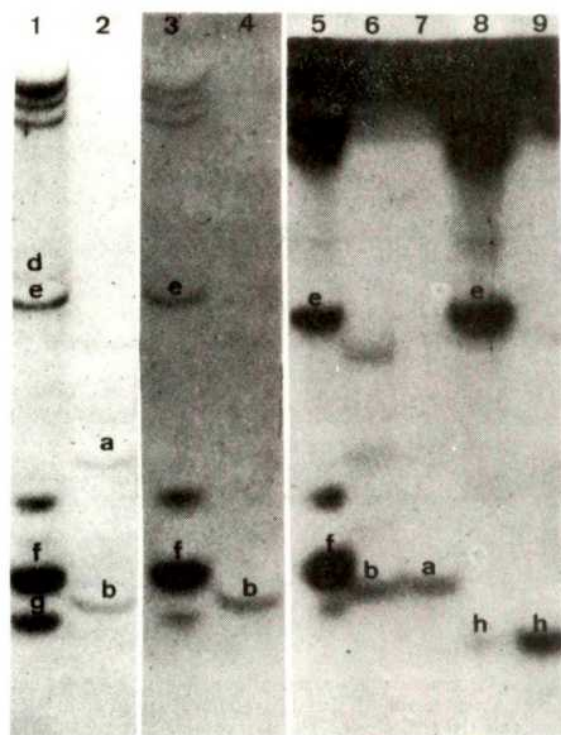
DNA fragments from *EcoRI*/*HindIII*, *EcoRI*/*PstI* and *HindIII*/*PstI* double digests were hybridised to  $^{32}\text{P}$ -labelled probes complementary to different regions of the ovalbumin mRNA (Fig. 4). As expected, multiple hybridisation bands were revealed by autoradiography. Their pattern was identical for oviduct and erythrocyte DNA (data not shown). Irrespective of the combination of restriction endonucleases, there was always one DNA fragment which hybridised strongly to *PstB*, but not to

*PstA* or *HinfA*: band 'b' for the *EcoRI*/*HindIII* digest (compare lanes 1, 4, 6, 8) and for the *HindIII*/*PstI* digest (lane 3, hybridisation with *PstA*, *PstB* and *HinfA* probes are not shown), band 'c' for the *EcoRI*/*PstI* digest (compare lanes 2, 5, 7 and 9). From the hybridisation pattern with *PstA* and *HinfA*, it is clear that fragments 'a' of *EcoRI*/*HindIII* and *EcoRI*/*PstI* double digests contain most of the ovalbumin sequences located to the left of the *PstI* site (see the map in Fig. 2a). But, when the lengths of these 'a' fragments were compared, a striking anomaly became apparent. The *EcoRI*/*HindIII* fragment was shorter than the *EcoRI*/*PstI* fragment by about 150 base pairs (2,600 and 2,750 base pairs, respectively). This shorter length cannot be attributed to a contaminating exonuclease activity in the *HindIII* enzyme, since all the restriction endonucleases used in this study were shown to be free of exonuclease activity even after prolonged incubations. Since the DNA fragment of an *EcoRI* digest which hybridises strongly to *PstA* or *HinfA* probes is 9,500 base pairs (Fig. 3, fragment 'a', lane 3), it follows that the *EcoRI*/*HindIII* and *EcoRI*/*PstI* fragment 'a' must share the same *EcoRI* terminus. There is therefore a *HindIII* site in the chromosomal *EcoRI*/*PstI* fragment 'a' at 150 base pairs from the *PstI* terminus. But, no *HindIII* site has been found in the cloned ovalbumin ds cDNA or in the *in vitro* synthesised ds cDNA (see above and Fig. 2). One has therefore to conclude that the *PstI* site present at one terminus of the DNA fragment 'a' of an *EcoRI*/*PstI* double digest is not identical to the *PstI* site present in the cloned or *in vitro* synthesised ds cDNA (Fig. 2). The simplest explanation to account for these results is that the DNA sequences complementary to the probes *PstA* (or *HinfA*) and *PstB* (Fig. 2) are physically separated, and that the DNA sequence coding for the



**Fig. 4** Detection of fragments containing sequences complementary to defined parts of ovalbumin mRNA in chicken DNA cleaved with a combination of restriction enzymes. Electrophoresis was on a 1% agarose gel.  $^{32}\text{P}$ -labelled fragments of *Hhaov* were prepared by the method described in Fig. 3. To obtain probes containing only sequences complementary to ovalbumin mRNA, labelled *Hhaov*, *PstA* and *PstB* were annealed to a 10-fold excess of ovalbumin mRNA at  $50.5^\circ\text{C}$  in 100 mM PIPES buffer, pH 7.9, containing 70% formamide, 100 mM NaCl, 10 mM EDTA (after denaturation at  $80^\circ\text{C}$  in this buffer). DNA-DNA hybrids are not stable in these conditions<sup>17</sup>. Single-stranded plasmid sequences and GC tails were digested with S1 nuclease (Miles). The RNA-DNA hybrid was precipitated with ethanol and then treated with RNase A in 10 mM Tris-HCl pH 8.0, 1 mM EDTA at  $50^\circ\text{C}$  to generate single-stranded probes. All other methods were as described in Fig. 3. Filters were hybridised to single-stranded *Hhaov* (lanes 1-3), single-stranded *PstA* (lanes 4 and 5), single-stranded *PstB* (lanes 6 and 7) or to *HinfA* (lanes 8 and 9). Lanes 1, 4, 6, 8, *EcoRI*/*HindIII* double digest of chicken DNA—sizes of the fragments are, in kilo base pairs: a, 2.6; b, 2.05; c, 1.8; d, 1.3. Lanes 2, 5, 7, 9, *EcoRI*/*PstI* double digest of chicken DNA—sizes of the fragments are in kilo base pairs: a, 2.75; b, 2.15; c, 1.9; d, 1.65; e, 1.3. Lane 3, *HindIII*/*PstI* double digest of chicken DNA—sizes in kilo base pairs: a, 4.4; b, 3.1; c, 1.5; d, 1.3. Fragment b does not hybridise to *PstA* or *HinfA*, but represents the main band with the *PstB* probe (not shown).





**Fig. 5** Detection of ovalbumin gene sequences in chicken DNA cleaved with *HinfI* and *HinfI* plus *HaeIII* restriction nucleases. Chicken DNA was digested with endonuclease *HinfI* (gift of Dr D. Duck) or *HaeIII* (Biolabs), with SV40 DNA as internal marker, and electrophoresed on a 1.5% Agarose gel. All other procedures were as described in Fig. 3. Autoradiography was for 3 d. Filters were hybridised to either  $^{32}\text{P}$ -Hhaov fragment,  $10^8$  c.p.m.  $\mu\text{g}^{-1}$  (lanes 1 and 2) or to  $^{32}\text{P}$ -HinfA fragment  $6 \times 10^7$  c.p.m.  $\mu\text{g}^{-1}$  (lanes 3–9). Lanes 1, 3 and 5, internal markers. Bands d and e are described in the legend to Fig. 1. Bands f and g correspond to the *HinfA* and *HinfB* fragments of Hhaov, 880 and 750 base pairs long, respectively (Fig. 2). Lanes 2, 4 and 6, chicken DNA digested with *HinfI*—sizes of the fragments in base pairs: a, 1,300; b, 800. Lane 7, chicken DNA digested with *HinfI* and *HaeIII*—size of band a, 800 base pairs. Lanes 8 and 9, internal markers; band e, see Fig. 1. fragment h, obtained by double digestion of Hhaov with *HaeIII* and *HinfI*, corresponds to the 650 base pair fragment between the *Hinf2* and *HaeIII* sites of the ds cDNA (see Fig. 2).

3'moiety of the mRNA (complementary to *PstA* and *HinfA*) is linked to a DNA sequence not coding for ovalbumin mRNA and containing a *HindIII* and a *PstI* site 150 base pairs apart.

Further evidence supporting the existence of a split ovalbumin gene was provided by the results of digestion of chicken DNA with *HinfI* endonuclease. As shown in Fig. 2, *HinfI* cuts the double-stranded DNA sequence complementary to ovalbumin mRNA in four places. One centrally located fragment, *HinfA*, is 880 base pairs long. This fragment should be found in a *HinfI* digest of chicken oviduct DNA, if the ovalbumin gene were not split in that region. Figure 5 shows the hybridisation pattern of the *HinfI* oviduct DNA fragments to  $^{32}\text{P}$ -labelled Hhaov and *HinfA* probes. The Hhaov probe hybridised mainly to two DNA fragments, 800 and 1,300 base pairs (Fig. 5, lane 2), while *HinfA* hybridised only to the 800-base pair DNA fragment (Fig. 5, lanes 4 and 6). The use of a *HinfI* digest of the Hhaov fragment (Fig. 5, lanes 1, 3 and 5) as an internal marker for hybridisation and molecular weight determination demonstrates unambiguously that the 80-base pair difference between the length of the *HinfA* fragment and that of its chromosomal DNA counterpart is real. In addition, the 800-base pair chicken DNA *HinfI* fragment which hybridises to the *HinfA* probe was not cut by *HaeIII* restriction endonuclease as shown in Fig. 5, lane 7, where a *HaeIII*/*HinfI* double digest of chicken DNA was electrophoresed. The implication of these results is that there is an interruption in the chromosomal DNA sequence coding for ovalbumin mRNA between the *Hinf2* and *HaeIII* sites of the ds cDNA (see Fig. 2).

## The ovalbumin gene is split in the coding region irrespective of the differentiated state of the cell

The data presented above show that in the chicken genome the DNA sequences coding for ovalbumin mRNA are split between the *Hinf2* and the *HaeIII* sites present in the ds cDNA. If the genomic DNA sequences coding for the remaining 5' part of the ovalbumin mRNA were in one piece one would expect that the *PstA* probe would hybridise to the same *EcoRI* or *HindIII* chicken DNA fragments which hybridise to the *PstB* probe, as these fragments would carry at least 320 bp also present in the *PstA* probe. As shown in Fig. 3, this was clearly not the case: the *EcoRI* and *HindIII* fragments 'b' which hybridised to the *PstB* probe did not hybridise to the *PstA* probe (Fig. 3). In consequence the genomic DNA sequences coding for ovalbumin mRNA must be split a second time near the *PstI* site of the ovalbumin ds cDNA (Fig. 2). The approximate location of the interruptions of the DNA sequences coding for ovalbumin mRNA are shown schematically in Fig. 6a, with respect to the restriction endonuclease sites present in the cloned ovalbumin ds cDNA (Fig. 2). Preliminary results suggest that the site of the first interruption lies close to the *HaeIII* restriction site. In any case at least one of the interruptions is located in a region coding for the ovalbumin protein: 1,161 nucleotides are required to code for the 387 amino acids of hen ovalbumin<sup>18</sup>, and it was recently shown that the first 250 nucleotides from the 3' end of the mRNA do not code for the protein<sup>15</sup>.

The occurrence of two interruptions could account for the origin of three of the fragments hybridising to the ovalbumin probe in an *EcoRI* digest of chicken DNA. The existence of a fourth band (Fig. 1) might suggest the presence of a third interruption.

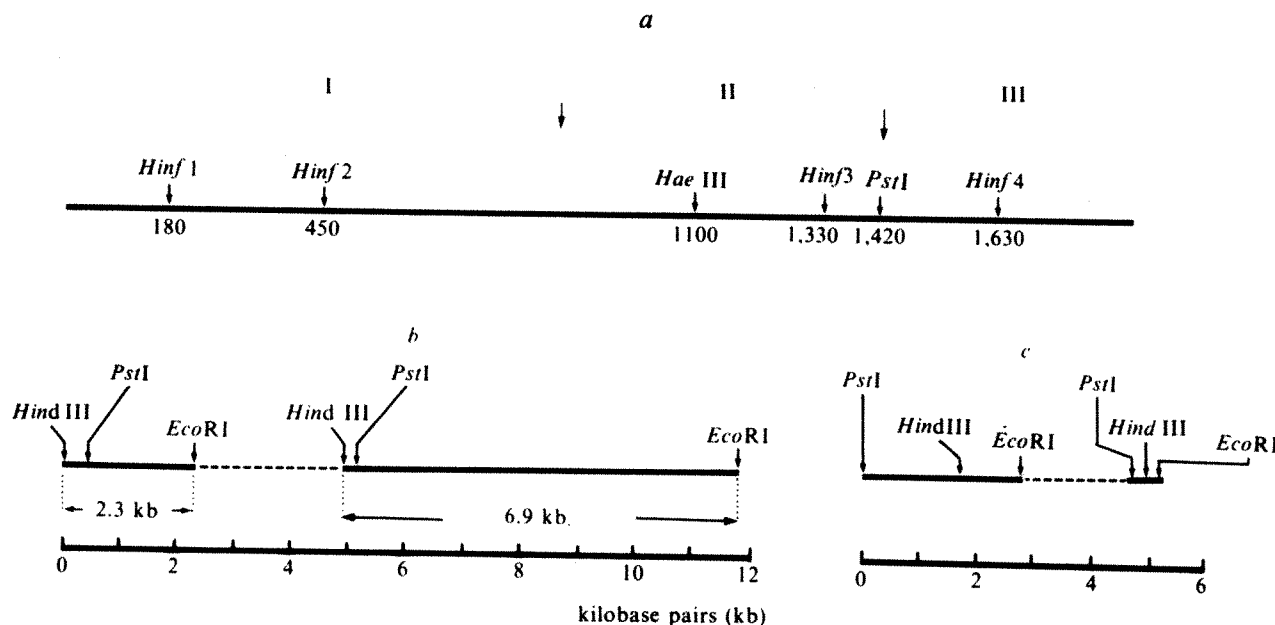
Figure 6b and c show the map of the restriction endonuclease sites flanking regions I and III, which code for RNA sequences present in the 3' and in the 5' regions of the ovalbumin messenger, respectively. These maps were established from the lengths of the main fragments hybridising to the *PstA* and *PstB* probes (Figs 1, 3 and 4). We do not yet know the orientation of transcription of the ovalbumin RNA sequences. Comparison of these maps indicates that the DNA sequences surrounding region III (Fig. 6c) cannot be included in the 12,000-base pair DNA fragment containing region I (Fig. 6). Depending on the orientation of transcription, region I is separated in the genome from region III by either at least 2,500 base pair or at least 7,500 base pair (see Fig. 6b).

Our hybridisation studies do not reveal any DNA fragment which could be derived from an uninterrupted 'intact' ovalbumin gene. In addition we have no evidence for major variations in the structural organisation of the split gene. Since 80% of the laying hen oviduct cells are actively engaged in ovalbumin mRNA synthesis<sup>3,4</sup> and since there is evidence that ovalbumin genes are present at approximately one copy per haploid genome (see ref. 3), we conclude that ovalbumin mRNA is transcribed from split genes. The oviduct DNA used in this study was isolated from total oviduct and it is therefore unlikely that loss of possible extrachromosomal elements carrying an 'intact' ovalbumin gene could have occurred. This peculiar arrangement of the genomic DNA coding for ovalbumin mRNA is apparently unrelated to the highly differentiated state of laying hen oviduct tubular cells. In all cases studied, the hybridisation patterns were identical for restriction digests of oviduct, erythrocyte or 5-d old embryo DNAs, indicating that there is no major modification of the ovalbumin gene organisation and environment during the differentiation of cells highly specialised in ovalbumin synthesis.

## Conclusions

It is not known how the different DNA regions of the split ovalbumin gene are organised in chicken DNA. There are two types of model that would be consistent with our results. In the first type of model, the insertion model, the different regions I, II and III (Fig. 6a) are all on the same DNA segment in the same order and orientation as in the *in vitro* synthesised ds cDNA, but they are separated by DNA sequences (insertions) that are not represented in mature mRNA. In the second type of model, the





**Fig. 6** Organisation of the sequences coding for ovalbumin mRNA in cellular DNA. *a*, Interruptions in the genomic ovalbumin DNA sequences. The arrows indicate the approximate location of the two interruptions and define three different regions (I, II and III) coding for ovalbumin mRNA. *b* and *c*, Physical map of restriction endonuclease cleavage sites flanking regions I and III, respectively. The dashed lines represent the DNA segments containing regions I or III. The sizes of the fragments used to establish these maps are (in kilo base pairs, see also Figs 1, 3 and 4) for region I: *EcoRI*, 9.5; *HindIII*, 4.9; *PstI*, 4.7; *EcoRI/HindIII*, 2.6; *EcoRI/PstI*, 2.75; *HindIII/PstI*, 4.4; for region III: *EcoRI*, 2.35; *HindIII*, 3.2; *PstI*, 4.7; *EcoRI/HindIII*, 2.05; *EcoRI/PstI*, 1.9; *HindIII/PstI*, 3.1. In each case the map was constructed using the first five values. From these maps one can predict sizes for the *HindIII/PstI* fragments which are in very good agreement with those found experimentally.

different regions are not in the same order and orientation as in the ds cDNA and could even be on different chromosomes. Further mapping of the ovalbumin sequences in chicken DNA is in progress, in the hope of finding a fragment which will contain all three regions of the ovalbumin gene, thereby supporting the insertion model. Up to now, no such fragment has been found. This does not rule out the insertion model, since the chance of finding such a fragment decreases with the length of the insertion.

The existence of DNA insertions within eukaryotic genes has recently been demonstrated for 28S ribosomal cistrons of *Drosophila melanogaster*<sup>19-22</sup>. But, since intact *Drosophila* ribosomal cistrons were also found, the physiological relevance of the insertion could be questioned. A 93-base pair insertion was detected and sequenced in the 5' region of the protein coding sequence of a cloned immunoglobulin variable gene of mouse embryo (S. Tonegawa, personal communication), and a 1,250-base pair insertion was found within the coding sequence of a cloned mouse plasmacytoma  $\lambda$  chain DNA, separating the sequences coding for the variable and constant regions of the  $\lambda$  chain (C. Brack & S. Tonegawa, personal communication). Using an approach very similar to ours, which eliminates the possible complications related to accidental selection of defective genes and/or DNA rearrangements during cloning, A. Jeffreys and R. Flavell (*Cell*, in the press) have detected the presence of a 600-base pair insertion within the protein sequence of the rabbit  $\beta$ -globin gene. A similar insertion was found in a cloned mouse  $\beta$ -globin gene (P. Leder, personal communication). It seems therefore that the splitting of genes within the protein coding sequence may have some generality in eukaryotic cells.

It is too early to speculate on the role of the split gene organisation in the regulation of gene expression at the transcriptional and/or post-transcriptional levels. But, the two types of model discussed above for the ovalbumin gene lead to different predictions concerning the general processes by which a split gene could be transcribed to give a single continuous mRNA. In the case of the insertion model three possible mechanisms already proposed to account for the mosaic late adenovirus mRNAs<sup>23-25</sup> could apply to the ovalbumin gene: (1) RNA polymerase B which is responsible for ovalbumin mRNA synthesis<sup>4</sup> could jump with the attached nascent RNA across looped out insertions, transcribing in an ordered fashion the ovalbumin gene regions III, II

and I (Fig. 6a) to yield directly the mRNA; (2) both insertions and messenger coding regions could be transcribed resulting in an ovalbumin mRNA precursor. The mature mRNA could then be formed by excision of the insertion transcripts and splicing of the mRNA sequences. The size of the precursor should be at least twice that of mature ovalbumin mRNA, since the minimum distance separating regions I and III in the insertion model is about 2,500 base pairs (see above and Fig. 6); (3) each of the mRNA coding segments may be transcribed independently and the ovalbumin mRNA generated by ligation of the transcripts. This latter mechanism, but not the two former ones, could also apply to the second type of model of the split gene where the mRNA coding regions are not placed in an ordered fashion (see above). We have no direct evidence to distinguish between these three possibilities, since the previous failure to detect an ovalbumin mRNA precursor<sup>26</sup> must be reinvestigated in the light of the recent finding concerning the short half-life of the  $\beta$ -globin mRNA precursor<sup>27,28</sup>.

The split gene type of organisation poses additional problems for studying *in vitro* the mechanisms involved in the specificity of transcription. The phenomenon of split genes in eukaryotic cells also diminishes the chance of obtaining single clones containing the DNA sequences coding for the entire mRNA and its putative regulatory elements. Furthermore, even where such clones may be isolated, it is unlikely that they could be used for the expression of eukaryotic genes in bacteria, as the necessary machinery for post-transcriptional processing may well not be present in prokaryotic organisms. Progress in this field may have to be based on the linking of known bacterial regulatory elements to ds cDNA clones containing the entire protein coding sequence.

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1. Hozumi, N. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3628-3632 (1976).
2. Oka, T. & Schimke, R. T. *J. cell. Biol.* **43**, 123-132 (1969).
3. Palmiter, R. D. *Cell* **4**, 189-197 (1975).

4. Bellard, M., Gannon, F. & Chambon, P. *Cold Spring Harb. Symp. quant. Biol.* **42**, (in the press).
5. Botchan, M., Topp, W. & Sambrook, J. *Cell* **9**, 269-287 (1976).
6. Southern, E. M. *J. molec. Biol.* **98**, 503-518 (1975).
7. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).
8. Humphries, P. *et al. Nucleic Acids Res.* **4**, 2389-2406 (1977).
9. Monahan, J. J., Woo, S. L. C., Liarakos, C. D. & O'Malley, B. W. *J. biol. Chem.* **252**, 4722-4728 (1977).
10. Blin, N. & Stafford, D. W. *Nucleic Acids Res.* **3**, 2303-2308 (1976).
11. Gross-Bellard, M., Oudet, P. & Chambon, P. *Eur. J. Biochem.* **36**, 32-38 (1973).
12. Denhardt, D. *Biochem. biophys. Res. Commun.* **23**, 641-646 (1966).
13. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1184-1189 (1975).
14. Philipson, L., Petterson, U. & Lindberg, U. *Molecular Biology of Adenovirus*, Virology Monographs **14**, (Springer, Berlin, 1975).
15. Brownlee, G. G. & Cartwright, E. M. *J. molec. Biol.* **114**, 93-117 (1977).

16. Maxam, A. A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560-564 (1977).
17. Casey, J. & Davidson, N. *Nucleic Acids Res.* **4**, 1539-1552 (1977).
18. Woo, S. L. *et al. J. biol. Chem.* **250**, 7027-7039 (1975).
19. Glover, D. M. & Hogness, D. S. *Cell* **10**, 167-176 (1977).
20. White, R. L. & Hogness, D. S. *Cell* **10**, 177-192 (1977).
21. Wellauer, P. K. & Dawid, I. B. *Cell* **10**, 193-212 (1977).
22. Pellegrini, M., Manning, J. & Davidson, N. *Cell* **10**, 213-224 (1977).
23. Berget, S. M., Moore, C. & Sharp, P. A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3171-3175 (1975).
24. Sambrook, J. *Nature* **268**, 101-104 (1977).
25. Chambon, P. *Cold Spring Harb. Symp. quant. Biol.* **42**, (in the press).
26. McKnight, G. S. & Schimke, R. T. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4327-4331 (1974).
27. Bastos, R. N. & Aviv, H. *Cell* **11**, 641-650 (1977).
28. Curtis, P. J., Mantei, N., Van den Berg, J. & Weissmann, C. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3184-3188 (1977).

# letters to nature

## Low energy $\gamma$ -ray observation of NGC4151

OBSERVATIONS of the Seyfert galaxy NGC4151 by Ariel V, OSO VII and UCSD (refs 1-3 respectively) have demonstrated that it has a relatively flat X-ray spectrum up to photon energies greater than 100 keV. The X-ray luminosity of this object is of the order of  $4 \times 10^{43}$  erg s $^{-1}$  if a distance of 20 Mpc is assumed, and exceeds the integrated luminosity at all the other observed greater wavelengths. Long-term observations<sup>4</sup> by Ariel V indicate that the X-ray emission may originate from a compact object with dimensions less than  $8 \times 10^{15}$  cm, presumably the nucleus of the Galaxy. Here we present the preliminary results of a positive measurement in the low energy  $\gamma$ -ray region of the spectrum, which are in good agreement with the X-ray data

and confirm the intense high energy luminosity of the object.

The Seyfert galaxy NGC4151 was one of the celestial objects observed during a recent balloon flight, from Palestine Texas on 22 and 23 May 1977, of the MISO low energy (0.2-20 MeV)  $\gamma$ -ray telescope<sup>5,6</sup>. The instrument is a semiactively-shielded Compton coincidence system having a sensitive area of about 500 cm $^2$  and an aperture of  $3^\circ \times 20^\circ$  FWHM. The gondola was steered by an orientation system designed to point the telescope in both zenithal and azimuthal planes. A hard X-ray telescope operating in the 30-250 keV energy range was also incorporated in the scientific payload. This detector viewed the same region of the sky as the  $\gamma$ -ray device, with an aperture of  $2^\circ \times 5^\circ$  FWHM and was designed around a sodium iodide crystal 3 mm thick and having a sensitive area of about 70 cm $^2$ .

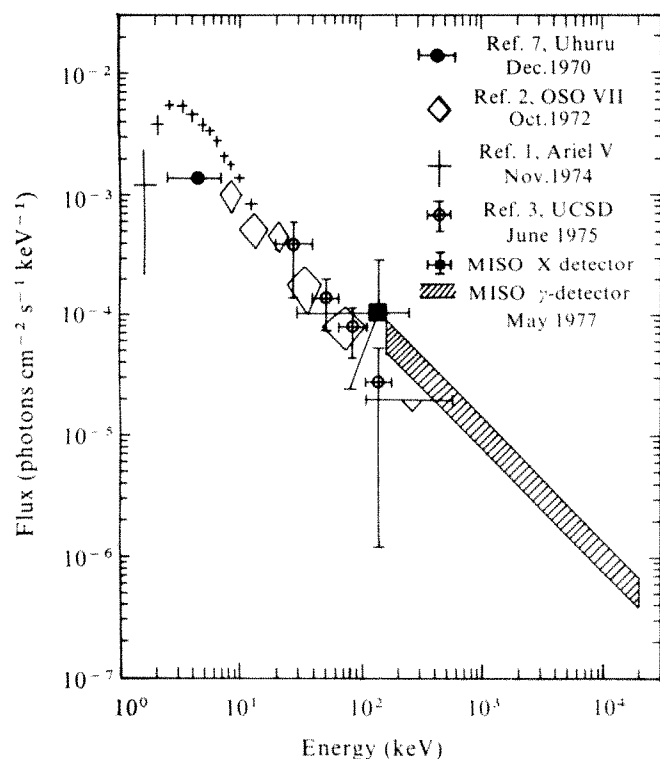
The entire observation of NGC4151 was made within  $18^\circ$  of the zenith at a floating altitude of about 7 mbar over a period of 3 h. Half of this time was used to obtain the background evaluation. NGC4151 was detected at the  $6.5\sigma$  level (based on the errors of the differences of the on and off source counting rate) in the 0.15-20 MeV energy band. In this preliminary analysis only real time counting rate data, processed by an on-line computer, from the sodium iodide crystal (component of the Compton coincidence system) were used. No spectral information is available at this stage. The observed counting rates after background subtraction at the same zenith angle were corrected for any changes in float altitude. No significant variation in background counting rate with azimuthal angle was observed.

Four transits of the source through the fine aperture of the telescope collimator were performed and a positive increase in  $\gamma$ -ray events observed in each case. The excess photon counting rate of  $8.4 \pm 1.3$  counts s $^{-1}$  at  $\gamma$ -ray energies and  $0.31 \pm 0.23$  counts s $^{-1}$  at X-ray wavelengths has been interpreted as a continuation of the previously measured hard X-ray spectrum. The  $\gamma$ -ray excess has been converted to a photon spectrum at the top of the atmosphere using conversion factors calculated for an input spectral index  $-1$ .

The  $\gamma$  flux obtained in this way is shown in Fig. 1 together with relevant soft and hard X-ray data. The hatched area takes into account both the uncertainties derived from statistical fluctuations at  $1\sigma$  level and the lack of knowledge of the precise exposure of the source. This latter uncertainty is caused by not having continuously updated orientation data in the quick-look mode to better than  $1^\circ$ .

The  $\gamma$ -ray results show good agreement with a power law extension of the X-ray data using a spectral index similar

**Fig. 1** The soft and hard X-ray measurements of NGC4151 compared with the MISO X and  $\gamma$  measurements. The integral  $\gamma$ -ray counting rate has been converted into a differential spectrum (hatched area) using a spectral index  $-1$ .



to that suggested by Baity *et al.*<sup>2</sup>. The extension of the emission spectrum of NGC 4151 up to 20 MeV would correspond to a luminosity of  $(8 \pm 2) \times 10^{45}$  erg s<sup>-1</sup> for a distance of 20 Mpc.

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1. Ives, J. C., Sanford, P. W. & Penston, M. V. *Astrophys. J. Lett.* **207**, L159–L162 (1976).
2. Baity, W. A., Jones, T. W., Wheaton, Wm. A. & Peterson, L. E. *Astrophys. J. Lett.* **199**, L5–L8 (1975).
3. Paciesas, W. S., Mushotzky, R. F. & Pelling, R. M. *Mon. Not. R. astr. Soc.* **178**, 23P–25P (1977).
4. Elvis, M. *Mon. Not. R. astr. Soc.* **177**, 7P–12P (1976).
5. Maccagni, D., Paizis, C., Perotti, F., Stiglitz, R., Villa, G., Railey, P., Dean, A. J. & Ramsden, D. *Proc. 9th Esab Symp. Frascati* 185–193 (1974).
6. Maccagni, D., *et al.* *14th Int. Cosmic Ray Conf. Munich* **9**, 3135–3140 (1975).
7. Gursky, H., Kellogg, E. M., Leong, C., Tananbaum, H. & Giacconi, R. *Astrophys. J. Lett.* **165**, L43–L48 (1971).

## Optical candidates for two X-ray bursters and an X-ray pulsar

WE suggest faint ( $B \sim 18$ ), blue stars as the optical counterparts of two X-ray bursters, 4U1636–53 (MXB1636–53)<sup>1–4</sup> and 4U1735–44 (MXB1735–44; KGX345–67)<sup>1,4–6</sup>, and for the 7-s X-ray pulsar, 4U1626–67<sup>1,7,8</sup>. The candidate stars have large ultraviolet excesses and were discovered well within the 20" and 30" X-ray error radii determined using the rotating modulation collimator experiment aboard the SAS-3 X-ray Observatory<sup>9,10</sup>. Photographic observations of several X-ray error boxes were performed (by C.R.C. and W.A.H.) in April 1976 (before final SAS-3 X-ray positions were known) with the 4-m telescope at the Cerro Tololo Interamerican Observatory (CTIO).  $R$ ,  $B$  and  $U$  plates were obtained for the regions including 4U1636–53 and 4U1735–44. The  $R$  plates (RG610 filter with 127-04 emulsion) are shown as

finding charts in ref. 9. Preliminary iris photometry of some of the plates was performed to identify the bluest stars in each error box for further study.

The photoelectric photometry was performed (by J.E.M.) using the 1.5-m telescope at CTIO during 8–13 June, 1977. A 10" aperture, a standard  $UBV$  filter set and an RCA 4516 photomultiplier tube were used during the entire run. For each colour typical integration times were 10 s for standard stars and 50 s for the programme stars and sky background. All objects were observed in the colour sequence  $BVUB$  as a check on sky conditions, telescope tracking and the stability of the instrumentation. The observations were made between the fourth quarter and new moon. The sky conditions were of photometric quality throughout each night with the exception of 9 June, which had some cirrus. Five or six  $UBV$  standard stars<sup>11</sup> were observed several times each night. A television guider, mounted at the customary position of the offset-guider eyepiece, was used on four nights (8, 9, 12 and 13 June), and provided the crucial capability to centre stars as faint as  $B \sim 20$  in the photometer aperture.

About 100 faint ( $B \sim 15$ –20) stars in the fields of 16 X-ray sources were examined during the six nights. Most of the observations were made within the 20–30" radius error circles determined using SAS-3 data. Stars with large ultraviolet excesses were discovered in the fields of three X-ray sources: 4U1626–67, 4U1636–53 and 4U1735–44.

A summary of the photometry data, coordinates of the stars and references to literature containing finding charts are given in Table 1. A measurement of the candidate for 4U1626–67 made while setting up on 12 June (0553 UT) and not included in Table 1 indicates that the star may be variable on timescales of minutes, but further observations are necessary to establish this. The three measurements of the candidate for 4U1735–44 show no evidence of variability during the course of four nights. Iris photometry of two  $R$  plates taken four days apart in April 1976 also shows no variability. A spectrogram of this candidate obtained by Bond<sup>12</sup> shows a generally featureless blue continuum with weak  $H\beta$  emission.  $\text{HeII } \lambda 4,686$  and  $\text{HeI } \lambda 5,875$  may also be present weakly in emission.

Figure 1 is a colour-colour diagram showing the measured locations of the candidate stars and several established optical counterparts (see refs 13–17), and the calculated location of a model accretion disk around a massive black hole<sup>18</sup>. The candidates for 4U1626–67 and 4U1636–53 lie further from the reddened supergiant or main sequence than any known counterparts and have colours inconsistent with those of a reddened, normal star. The candidate for 4U1735–44 has the colours of Sco X-1 as well as the colours of a late O or B0 star suffering  $\sim 1$  mag of extinction.

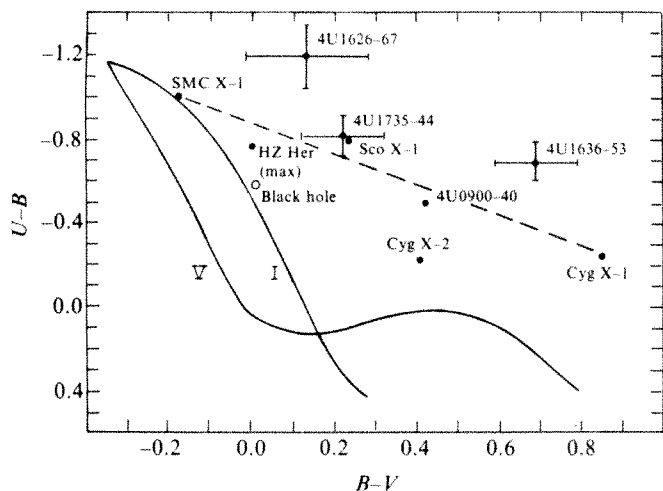
Table 1 Description of optical candidates

X-ray source	Star no.*	Star position ( $\pm 3''$ )		UT (June 1977)	$V^\dagger$	$B-V^\dagger$	$U-B^\dagger$
		RA (1950) ( $h^m, s^s$ )	Dec. (1950)				
4U1626–67 (2S1627–673)	4	16h27m14.2s (321.8–13.1)	–67°21'16"	12d06h05m	18.67	+0.00	–1.18
				12 06 34	18.42	+0.26	–1.23
4U1636–53 (2S1636–536)	3	16 36 56.2 (332.9–4.8)	–53°39'15"	12 03 21	17.52	+0.69	–0.70
4U1735–44 (2S1735–444)	5	17 35 19.0 (346.1–7.0)	–44°25'19"	09 06 39	17.43	+0.24	–0.78
				11 05 10 13 06 54	17.40 17.52	+0.22 +0.21	–0.86 –0.81

\*Finding charts showing the SAS-3 (2S) positions and labelled with these star numbers are given for 4U1626–67 (in ref. 10) and for 4U1636–53 and 4U1735–44 (in ref. 9).

†Uncertainties ( $\sim 90\%$  confidence) in  $V$ ,  $B-V$  and  $U-B$  are  $\pm 0.15$  mag. for 4U1626–67 and  $\pm 0.10$  mag. for 4U1636–53 and 4U1735–44. Uncertainties due to counting statistics are  $\lesssim \pm 0.05$  mag.

The candidates for 4U1735-44 and 4U1636-53 bring the number of possible stellar counterparts to X-ray burst sources to three (the third is Davidson's candidate for Ser X-1<sup>19,20</sup>). The colours of the two presented here are far from those estimated by Katz<sup>18</sup> for an accretion disk surrounding a massive black hole. Evidence of the binary nature of any or all these candidates could resolve the uncertainty over the nature of the bursters (see refs 21, 22). The extreme colours of the candidate for 4U1626-67 are remarkable since this source is a rapid X-ray pulsar<sup>8</sup>. The optical emission of all other X-ray pulsars, with the exception of HZ Her, is dominated by that of the OB companion.



**Fig. 1** A colour-colour diagram in the  $UBV$  system showing the three candidates presented in this paper, several well-established optical counterparts<sup>13-17</sup> and a model accretion disk surrounding a massive black hole<sup>18</sup>. The solid curves give the locus of colours for unreddened, main-sequence stars (V) and supergiants (I) following Davis.<sup>24</sup> The dashed curve is the reddening line for a B0 supergiant.

Possibly 4U1626-67 is an even more extreme example of the HZ Her phenomenon, in which the optical luminosity at maximum light is a direct result of the X-ray emission (see ref. 23).

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1. Forman, W. *et al.* *Astrophys. J. Suppl.* (in the press).
2. Swank, J. H., Becker, R. H., Pravdo, S. H., Saba, J. R. & Serlemitsos, P. J. *IAU Circ. No. 3000* (1976).

3. Hoffman, J. A., Lewin, W. H. G. & Doty, J. *Astrophys. J. Lett.* (submitted).
4. McClintock, J. E. *IAU Circ. No. 3084* (1977).
5. Sagdeev, R. Z. & Melioranskij, A. S. *IAU Circ. No. 2959* (1976).
6. Lewin, W. H. G., Hoffman, J. A., Doty, J., Li, F. K. & McClintock, J. E. *IAU Circ. No. 3075* (1977).
7. McClintock, J. E. *IAU Circ. No. 3088* (1977).
8. Rappaport, S. *et al.* *Astrophys. J. Lett.* (submitted).
9. Jernigan, J. G., Apparao, K. M. V., Bradt, H. V., Doxsey, R. E. & McClintock, J. E. *Nature* **270**, 321-323 (1977).
10. Bradt, H. V. *et al.* *Nature* **269**, 496-497 (1977).
11. Landolt, A. U. *Astr. J.* **78**, 959-981 (1973).
12. Bond, H. E. *IAU Circ. No. 3085* (1977).
13. Hiltner, W. A. & Mook, D. E. *A. Rev. Astr. Astrophys.* **8**, 139-160 (1970).
14. Murdin, P. & Webster, B. L. *Nature* **233**, 110 (1971).
15. Hiltner, W. A., Werner, J. & Osmer, P. *Astrophys. J. Lett.* **175**, L19-L22 (1972).
16. Webster, B. L., Martin, W. L., Feast, M. W. & Andrews, P. J. *Nature phys. Sci.* **240**, 183 (1972).
17. Boynton, P. E., Canterna, R., Crosa, L., Deeter, J. & Gerend, D. *Astrophys. J.* **186**, 617-624 (1973).
18. Katz, J. I. *Astrophys. Lett.* **18**, 69-72 (1977).
19. Davidson, A. *IAU Circ. No. 2824* (1975).
20. Doxsey, R. E., Apparao, K. M. V., Bradt, H. V., Dower, R. G. & Jernigan, J. G. *Nature* **269**, 112-116 (1977).
21. Lewin, W. H. G. *et al.* *Nature* **267**, 28-30 (1977).
22. Ostriker, J. P. paper presented at 8th Texas Symp. Relativistic Astrophys. Boston (1976).
23. Joss, P. C., Avni, Y. & Rappaport, S. *Astrophys. J.* (submitted).
24. Davis, R. J. *Astrophys. J.* **213**, 105-110 (1977).

## Positions of three X-ray burst sources

PRECISE (20-30'') positions of three steady X-ray sources which have been identified recently as X-ray burst sources 4U1636-53 = MXB1636-53 (refs 1, 2), 4U1728-33 = MXB1728-34 (refs 3, 4), and 4U1735-44 = MXB1735-44 (refs 5, 6, 7) are reported here. These positions, hereafter designated with a 2S prefix, have been derived from data obtained with the SAS-3 rotating modulation collimators during a survey of the galactic plane<sup>8-10</sup>. The small solid angles of the error regions make possible a thorough survey of all potential optical, infrared, and radio counterparts of these burst sources. Two of these positions, 2S1636-536 and 2S1735-444, have led to the probable identifications of optical counterparts<sup>11</sup>.

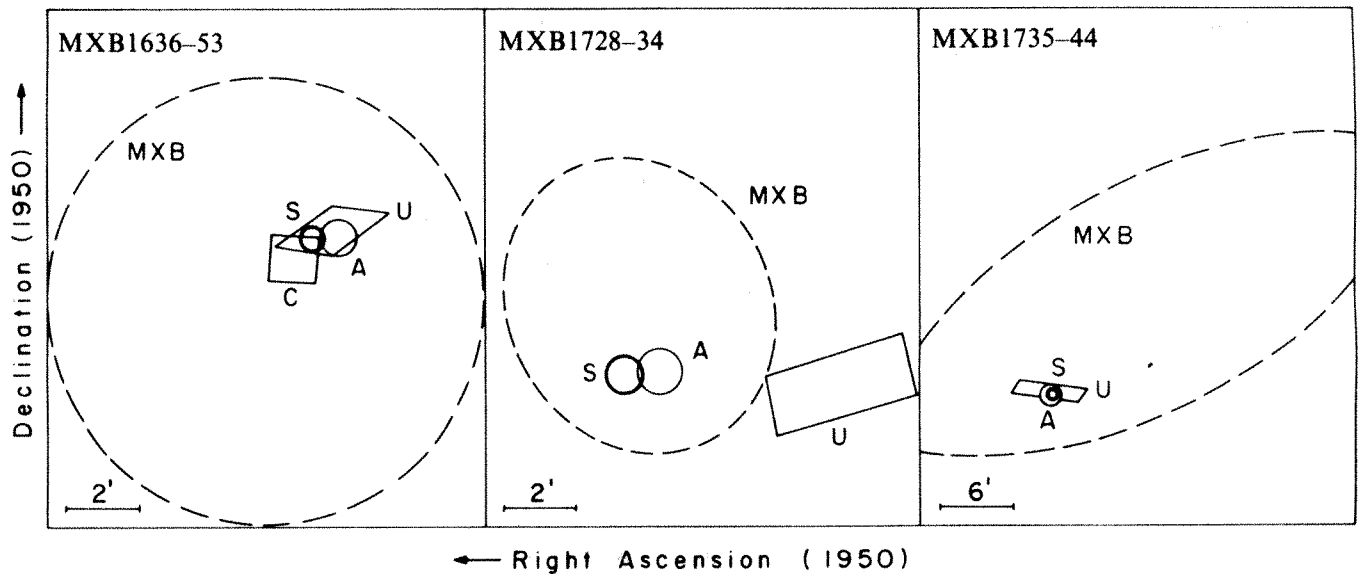
The burst sources MXB1636-53 and MXB1728-34 were discovered respectively with the OSO-8 (ref. 1) and SAS-3 (refs 3, 12) satellites. The source MXB1735-44, located precisely<sup>5</sup> with SAS-3, is probably the burst source KGX345-6 that was discovered earlier<sup>6,7</sup> with the COSMOS 428 satellite. All three sources emit X-ray bursts with peak flux densities (2-15 keV) comparable to, or greater than, that of the Crab nebula and with rise times of 1-2 s.

The association of the X-ray burst sources with the previously known Uhuru sources is now well established (Fig. 1). These identifications have been made possible through the determination<sup>2,3,5</sup> of the burst source positions to an accuracy of 6-10' with the SAS-3 slat collimators<sup>13</sup>. Positions of the steady components of these sources have been measured with the Uhuru<sup>14</sup>, Copernicus<sup>15</sup>, and Ariel V<sup>16</sup> satellites. The present SAS-3 positions are shown in Figs 1 and 2 and Table 1. Astrometric positions and stellar magnitudes are given in Table 2.

An optical study based upon the Copernicus position for MXB1636-53 led to the suggested optical identification<sup>17</sup> of a flare star (star M in Fig. 2) that lies outside but near the 2S error region. A recent study of the 2S1636-536 and 2S1735-444 positions led to the discovery<sup>11</sup> of two faint  $V \sim 17.5$ , ultraviolet excess stars that lie well within the 2S regions (stars 3 and 5 respectively in Fig. 2). A search<sup>18</sup> for an optical counterpart for MXB1728-34 has yielded no likely candidates. Several bright ( $K < 10$  mag) infrared sources have been detected, however, within  $\sim 1'$  of 2S1728-337 by I. Glass and by S. Kleinmann (personal communications).

Altogether, we have measured with SAS-3 precise (20-35'') positions of six steady X-ray sources (other than known globular clusters) which have been identified<sup>1-7,13,19-22</sup> as





**Fig. 1** The burst source locations (dashed lines) and steady source locations (solid lines) MXB1636-53; MXB1728-34 and MXB1735-44. The burst source locations designated MXB, were obtained with the SAS-3 slit collimators (refs 2, 3, 5 and J. Hoffman, personal communication). The steady source locations are from the Uhuru 4U catalogue (U, ref. 14), the Ariel V rotating modulation collimator (A, ref. 16), the Copernicus satellite (C, ref. 15), and the SAS-3 rotating modulation collimator [S (dark circles) present work].

counterparts of X-ray bursters. Previously we reported<sup>9</sup> the positions of Ser X-1 (2S1837+049), A1905+00 (2S1905+000), and 4U1915-05 (2S1916-053). All six lie within 40° of the galactic centre and within 10° of the galactic plane. The intensities of the steady sources measured with SAS-3 agree roughly with those reported previously<sup>14,23</sup>. None of these sources is known to exhibit periodicities, eclipses, or rapid variability other than bursts. These featureless characteristics are typical of the X-ray sources which are located in the galactic bulge. We also note that the burst sources exhibit a galactic distribution<sup>24</sup> similar to the bulge sources.

The bulge X-ray sources are similar to the ~ 6 steady X-ray sources which are identified<sup>23,25-27</sup> with globular clusters, in

that they exhibit a deficiency of hard X rays and are associated with late-type stars in regions of high stellar density<sup>28,29</sup>. Four globular clusters, NGC6624 (ref. 30), NGC1851 (refs 31, 32), NGC6441 (ref. 33) and Liller 1 (refs 34, 35) are now believed to be X-ray bursters. The physical significance of these apparent similarities among burst sources, the bulge sources, and globular cluster sources is not well understood (see ref. 29). This makes the search for and study of optical, infrared, and radio counterparts of the burst sources particularly compelling.

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**Fig. 2** Finding charts for the SAS-3 positions. The charts for 2S1636-536 and 2S1735-444 are from R plates taken with the CTIO 4 m telescope by W. A. Hiltner and C. Canizares. The chart for 2S1728-337 is from the southern extension of the Palomar Sky Survey (National Geographic Society). North is up and east is to the left. The flare star designated M (2S1636-536) has been suggested<sup>17</sup> as an optical counterpart. The 2S1636-536 and 2S1735-444 regions each contain a star (numbers 3 and 5 respectively) with a pronounced ultraviolet excess<sup>11</sup>.

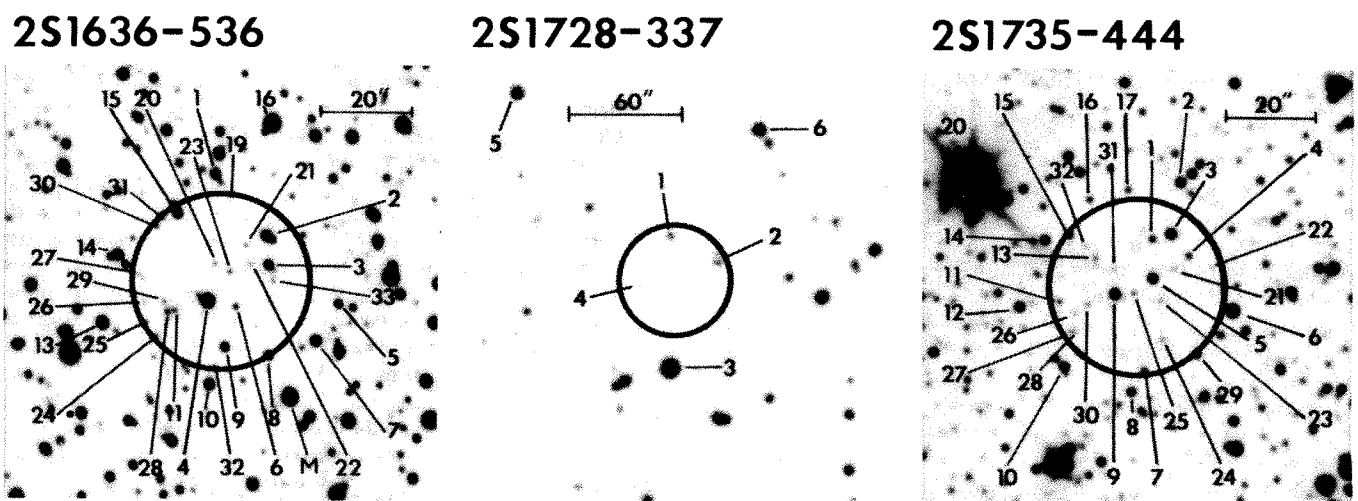


Table 1 Celestial positions

SAS-3 designation	Other designations*	Position (1950)		$l^{\text{II}}$ $b^{\text{II}}$	Error radius (90%)	Flux density† (2–11 keV)	Comments
		$\alpha$	$\delta$				
2S1636–536	4U1636–53	16h 36min 57.6s 249.2400°	–53°39'21'' –53.6558°	332.9° –4.8°	20''	240 $\mu\text{Jy}$	MXB1636–53
2S1728–337	GX354+0 4U1728–33	17 28 39.6 262.1650	–33 47 52 –33.7978	354.3 –0.2	30	95	MXB1728–34
2S1735–444	GX346–7 4U1735–44	17 35 19.5 263.8313	–44 25 22 –44.4228	346.1 –7.0	20	160	MXB1735–44 KGX345–6?

\*Refs 14, 36, 37.

†1.0  $\mu\text{Jy}$  corresponds to  $2.2 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$  (2–11 keV).  $I_{\text{crab}} = 1,060 \mu\text{Jy}$ . See refs 8, 9.

Table 2 Stellar astrometry and magnitudes

Source	Star	$m_v^*$	Position (1950)†	
			$\alpha$	$\delta$
2S1636–536	4	16.2		
	9	17.8		
	16		16 h 36 min 56.3 s	–53° 38' 45''
2S1728–337	2	18.2		
	3	13.5	17 28 39.7	–33 48 36
	5		17 28 46.1	–33 46 11
	6		17 28 35.9	–33 46 30
2S1735–444	3	17.6		
	20		17 35 22.9	–44 24 59

\* From photoelectric photometry<sup>11</sup>  $\pm 0.1 \text{ mag}$  ( $\pm 0.25 \text{ mag}$  for star 2).† Precise to  $< 3''$ .

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- Swank, J. H., Becker, R. H., Pravdo, S. H., Saba, J. R. & Serlemitsos, P. J. *IAU Circ.* No. 3000 (1976).
- Hoffman, J. A., Lewin, W. H. G. & Doty, J. *Astrophys. J. Lett.* (in the press); *IAU Circ.* No. 3025 (1977).
- Hoffman, J. A. *et al. Astrophys. J. Lett.* **210**, L13–L17 (1976).
- Carpenter, G. F., Skinner, G. K., Wilson, A. M. & Willmore, A. P. *Nature* **262**, 473–474 (1976).
- Lewin, W. H. G., Hoffman, J. A., Doty, J., Li, F. K. & McClintock, J. E. *IAU Circ.* No. 3075 (1977).
- Babushkina, O. P. *et al. Sov. Astr. Lett.* **1**, 32–34 (1975).
- Sagdeev, R. Z. *IAU Circ.* No. 2959 (1976).
- Bradt, H. V. *et al. Nature* **269**, 21–25 (1977).
- Doxsey, R. E., Apparao, K. M. V., Bradt, H. V., Dower, R. G. & Jernigan, J. G. *Nature* **269**, 112–116 (1977).
- Bradt, H. V. *et al. Nature* **269**, 496–497 (1977).
- McClintock, J. E., Canizares, C., Bradt, H. V., Doxsey, R., Jernigan, J. G. & Hiltner, W. A. H. *Nature* **270**, 320–321 (1977); *IAU Circ.* Nos 3084, 3088 (1977).
- Lewin, W. H. G. *IAU Circ.* No. 2922 (1976).
- Lewin, W. H. G. *et al. Mon. Not. R. astr. Soc.* **177**, 93p–100p (1976).
- Forman, W. *et al. Astrophys. J. Suppl.* (in the press).
- Willmore, A. P. *et al. Mon. Not. R. astr. Soc.* **169**, 7–23 (1974).
- Wilson, A. M., Carpenter, G. F., Eyles, C. J., Skinner, G. K. & Willmore, A. P. *Astrophys. J. Lett.* **215**, L111–L115 (1977).
- Murdin, P. *et al. Mon. Not. R. astr. Soc.* **169**, 25–34 (1974).
- Liller, W. *Astrophys. J. Lett.* **213**, L21–L23 (1977).
- Swank, J. H., Becker, R. H., Pravdo, S. H. & Serlemitsos, P. J. *IAU Circ.* No. 2963 (1976).
- Li, F. K. *et al. Mon. Not. R. astr. Soc.* **179**, 21p–25p (1977).
- Becker, R. H. *et al. Astrophys. J.* (in the press).
- Lewin, W. H. G., Hoffman, J. A. & Doty, J. *IAU Circ.* No. 3087 (1977).
- Seward, F. D., Page, C. G., Turner, M. J. L. & Pounds, K. A. *Mon. Not. R. astr. Soc.* **175**, 39p–46p (1976).
- Lewin, W. H. G. *et al. Nature* **267**, 28–30 (1977).
- Giacconi, R. *et al. Astrophys. J. Suppl.* **27**, 37–64 (1974).
- Clark, G. W., Markert, T. H. & Li, F. K. *Astrophys. J. Lett.* **199**, L93–L96 (1975).

- Markert, T. H., Backman, D. E., Canizares, C. R., Clark, G. W. & Levine, A. M. *Nature* **257**, 32–33 (1975).
- Canizares, C. *Astrophys. J.* **201**, 589–592 (1975).
- Markert, T. H. *et al. Astrophys. J.* (in the press).
- Grindlay, J. *et al. Astrophys. J. Lett.* **205**, L127–L130 (1976).
- Forman, W. & Jones, C. *Astrophys. J. Lett.* **207**, L177–L180 (1976).
- Clark, G. W. & Li, F. K. *IAU Circ.* No. 3092 (1977).
- Li, F. K. & Clark, G. W. *IAU Circ.* No. 3095 (1977).
- Liller, W. *IAU Circ.* No. 2929 (1976).
- Lewin, W. H. G. *et al. Astrophys. J. Lett.* **207**, L95–L99 (1976).
- Kellogg, E., Gursky, H., Murray, S., Tananbaum, H. & Giacconi, R. *Astrophys. J. Lett.* **169**, L99–L103 (1971).
- Heinz, C. *et al. IAU Circ.* No. 2466 (1972).

## Identification of the $\lambda 2,200\text{\AA}$ interstellar absorption feature

A BROAD absorption feature centred on  $\lambda 2,200\text{\AA}$  with a half-width of  $\sim 300\text{\AA}$  appears in the spectra of reddened stars<sup>1–3</sup>. This conspicuous feature in the interstellar extinction curve, might hold an important clue to the identity of a major component of interstellar matter, but it has defied identification for over a decade. Here we identify this band as representing the integrated effect of a set of bicyclic compounds, each with the empirical formula  $\text{C}_8\text{H}_6\text{N}_2$ . Such nitrogenated structures could form in stellar mass flows of the type which we have also discussed<sup>4</sup>. A significant mass fraction of all interstellar material might exist in this form.

Graphite particles have been considered the most plausible candidate for the  $\lambda 2,200\text{\AA}$  absorption feature. Whilst a small particle resonance in graphite can occur close to  $\lambda 2,200\text{\AA}$ , the central wavelength of this feature is sensitively dependent on particle shape<sup>5</sup>. Spherical particles with sizes small compared to the wavelength are necessary to produce agreement with observational data, but a more realistic distribution of shapes would produce a considerably broader absorption feature than is required. It therefore seems that a narrower molecular absorption must be superimposed on an underlying broader extinction hump which could be caused by extinction from graphite grains with a wide spread in their shapes.

We have discussed a possible molecular origin for the  $\lambda 2,200\text{\AA}$  band due to  $\pi \rightarrow \pi^*$  electronic transitions in a wide class of molecules involving conjugated double bonds<sup>6</sup>. We now limit our search to the nitrogenated heterocyclic compounds listed in Table 1. The first four compounds represent all possible arrangements of two N atoms in a bicyclic structure, with the hetero-atoms confined to one ring only. The fifth compound is an isomer where there is one N atom in each of the two rings.

An average molar absorptivity function  $\langle \epsilon(\lambda) \rangle$  was computed for these materials from available spectroscopic data<sup>7</sup>. A normalised absorptivity  $A(\lambda)$  given by

$$A(\lambda) = \frac{\langle \epsilon(\lambda) \rangle - \langle \epsilon(\lambda_0) \rangle}{\langle \epsilon(\lambda_1) \rangle - \langle \epsilon(\lambda_0) \rangle} \quad (1)$$

with  $\lambda_0^{-1} = 3.8 \mu\text{m}^{-1}$ ,  $\lambda_1^{-1} = 4.55 \mu\text{m}^{-1}$  is plotted in Fig. 1. Our computed curve for  $\text{C}_8\text{H}_6\text{N}_2$  isomers agrees exactly with the interstellar extinction data with respect to the central wavelength, but the 'average' interstellar band is apparently  $\sim 30\%$  wider. The latter departure could easily be ascribed to an

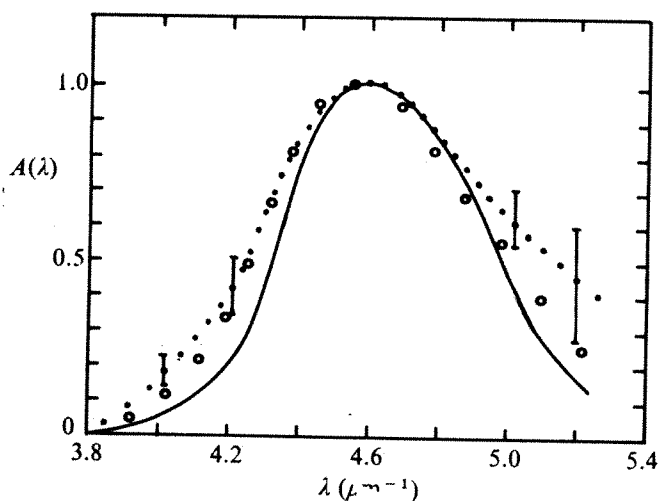


Fig. 1 Normalised average molar absorptivity for  $C_8H_6N_2$  isomers (solid curve) compared with interstellar extinction data in the waveband  $3.8 \mu m^{-1} < \lambda^{-1} < 5.4 \mu m^{-1}$ . Normalisation is to 0.0 at  $\lambda^{-1} = 3.8 \mu m^{-1}$ , 1.0 at  $\lambda^{-1} = 4.55 \mu m^{-1}$ . Vertical bars give indication of spread of astronomical data. Dotted curve is the mean extinction curve of Bless and Savage<sup>2</sup> normalised according to equation (1). Open circles give mean extinction ( $E(\lambda - V)/E(B - V)$ ) relative to extinction data for  $\theta$ -Orionis, and normalised as above.

underlying graphite particulate extinction (scattering+absorption) peak upon which the narrower molecular absorption band is superposed. Since  $\theta$ -Orionis shows a broad extinction hump centred on  $\lambda 2,200\text{\AA}$  rather than the sharper band which is more common, we can reasonably attribute this extinction curve to an underlying graphite component. The mean extinction curve relative to the extinction data for  $\theta$ -Orionis (Fig. 1) provides much closer agreement with the molecular absorption data, as we would expect. The mass density of molecules necessary to produce the observed strength of the  $\lambda 2,200\text{\AA}$  interstellar band ( $\sim 1.5 \text{ mag kpc}^{-1}$ ) is  $\sim 10^{-27} \text{ g cm}^{-3}$  implying that only  $\sim 10\%$  of interstellar C and N is in this form.

An identification of ring compounds of the type listed in Table 1 may have important consequences. Linear molecules such as HCN,  $HC_3N$ ,  $HC_5N$ ,  $HC_7N$  which have already been observed in interstellar space may result from the break-up of

Table 1 Properties of various isomers of  $C_8H_6N_2$

Compound	Structural formula	$\lambda_m(\text{\AA})$	$\epsilon$ (molar absorptivity)
Cinnoline		2,250	40,000
Quinazoline		2,220	35,500
Quinoxaline		2,340	23,400
Phthalazine		2,150	56,000
1,5 Naphthyridine		2,060	54,000

these more complex structures. It would now be worthwhile to search systematically for ring molecules by radioastronomical techniques.

Sakata *et al.*<sup>8</sup> have reported the detection of an absorption feature at  $\lambda 2,200\text{\AA}$  in soluble organic material extracted from the Murchison meteorite. In view of the possible connection of this material with interstellar matter, a chemical analysis of the

molecules responsible for the meteoritic  $2,200\text{\AA}$  band will also be valuable. It is interesting that several nitrogen heterocyclic compounds, including purines, pyrimidines and pyrroles have recently been identified in carbonaceous chondrites<sup>9-11</sup>.

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1. Stecher, T. P. *Astrophys. J.* **157**, L125 (1969).
2. Bless, R. C. & Savage, D. *Astrophys. J.* **171**, 193 (1972).
3. Witt, A. N. & Lillie, C. F. in *Scientific Results from the Orbiting Astronomical Observatory OAO-2* (ed. Code, A. (NASA SP-310, 1972)).
4. Hoyle, F. & Wickramasinghe, N. C. *Nature* (in the press).
5. Gilra, D. P. in *Scientific Results from the Orbiting Astronomical Observatory OAO-2* (ed. Code, A.) (NASA SP-310) (1972).
6. Hoyle, F. & Wickramasinghe, N. C. *Nature* **266**, 241-242 (1977).
7. *UV Atlas of Organic Compounds* (Butterworths, London, 1966).
8. Sakata, A. *et al.* *Nature* **266**, 241 (1977).
9. Hayatsu, R. *Science* **146**, 1291 (1964).
10. Hayatsu, R., Studier, M. H., Oda, A., Fuse, K. & Anders, E. *Geochim. cosmochim. Acta* **32**, 175 (1968).
11. Folsome, C. E., Lawless, J. G., Romiez, M. & Ponnampuram, C. *Geochim. cosmochim. Acta* **37**, 455 (1973).

## Has the Sun a companion star?

PULSARS are accurate timekeepers. They are believed to be rotating neutron stars, with strong magnetic fields, and the energy they radiate is at the expense of their rotational kinetic energy<sup>1</sup>. As each pulsar ages, its period  $P$  (relative to the Solar System barycentre) slowly increases, and its period derivative  $\dot{P} (= dP/dt)$  slowly decreases. Certain interesting pulsars (displayed in Table 1) have anomalously small period derivatives, and rather surprisingly, are found grouped together in the same region of the sky (shown in Fig. 1). I suggest here, as an explanation of the peculiar properties of these pulsars, that the barycentre of the Solar System is accelerated, possibly because the Sun is a member of a binary system and has a hitherto undetected companion star.

The characteristic spindown age of any pulsar is  $T = P/\dot{P}$ . Most pulsars are born close to the galactic plane and most pulsars have high velocities; hence, as they age, they disperse away from the galactic plane. By studying the observed height distribution of pulsars about the galactic plane, various authors<sup>1-5</sup> have drawn the following conclusions.

First, the 'young' pulsars of  $T \leq 3 \times 10^6 \text{ yr}$  (or  $\dot{P} \geq 10^{-14}$ ) have an observed height distribution that shows a rise in scale-height with increasing  $T$ , that is consistent with the known velocities, and the spindown age is a reasonable approximation of true age.

Second, most pulsars (57 out of 85) of known period derivatives<sup>5,6</sup> are 'middle-aged' and have spindown ages in the range  $3 \times 10^6 \leq T \leq 3 \times 10^8 \text{ yr}$  (or  $10^{-14} \geq \dot{P} \geq 10^{-16}$ ). In their case  $T$  is generally an unreliable indicator of true age, and at best is an upper limit. This is possibly because of magnetic-field decay<sup>1,2,8</sup>, or change in magnetic-field orientation<sup>8-10</sup>, or perhaps because of other unknown processes. The positions of these 57 pulsars in equatorial coordinates are shown in Fig. 1; they are distributed about the galactic equator and show concentration toward the galactic centre.

Third, the remaining five pulsars of  $T \geq 3 \times 10^8 \text{ yr}$ , listed in Table 1, have anomalously low period derivatives of  $\dot{P} < 10^{-16}$ . PSR1913+16 is the binary pulsar discovered by Hulse and Taylor<sup>11</sup>, and its low period derivative  $\dot{P} = 9 \times 10^{-18}$  may be due to exchange of angular momentum. There are, at present, no explanations for the anomalously low period derivatives of the remaining four pulsars.

The period derivatives shown in Table 1 must be further reduced by the Shklovsky<sup>16</sup> or 'train-whistle' effect (whistle-blowing trains passing in all directions through a nearby railway station have positive  $\dot{P}$ ). If  $\dot{P}_0$  is the intrinsic period derivative of a source

moving with transverse velocity  $v_t$  at distance  $d$ , and  $\dot{P}$  is the observed period derivative, then

$$\frac{\dot{P}}{P} = \frac{\dot{P}_0}{P} + \frac{v_t^2}{cd} \quad (1)$$

where  $c$  is the velocity of light. For PSR1944+17 the Shklovsky effect reduces  $\dot{P}_0$  to almost zero<sup>17</sup>; and for PSR1952+29 it is found that  $\dot{P}_0$  is negative<sup>15</sup> and the pulsar is apparently spinning up. Mansfield and Rankin<sup>15</sup> have investigated various mechanisms that might explain the negative intrinsic period derivative of PSR1952+29, and conclude that all hitherto proposed mechanisms are either inadequate or unlikely.

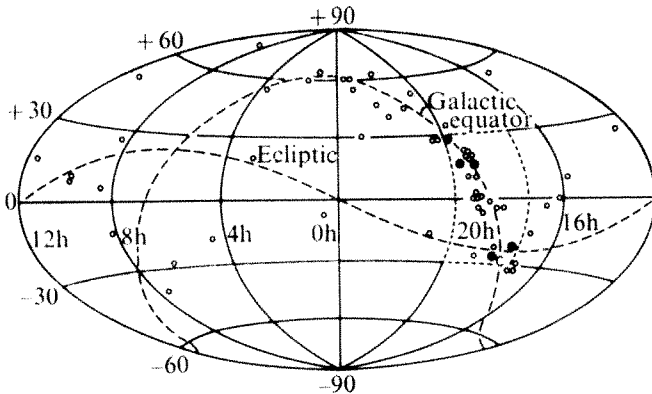


Fig. 1 Plot of pulsar positions in equatorial coordinates on an equal area projection. ○, Pulsars of  $10^{-14} > \dot{P} \geq 10^{-16}$ ; ●, pulsars of  $\dot{P} < 10^{-16}$ ; C, Galactic centre.

The pulsars of small period derivative in Table 1 are shown with their positions plotted in Fig. 1. It is remarkable that all five lie in the same region of the sky with their right ascension ( $\alpha$ ) and declination ( $\delta$ ) within the ranges  $17^{\text{h}}30' < \alpha < 19^{\text{h}}52^{\text{m}}$  and  $-27^\circ < \delta < +32^\circ$ . Inside the box (shown in Fig. 1), defined by these coordinate limits, are found 23 pulsars of which five have the anomalously small  $\dot{P}$ ; outside the box are the remaining 39 pulsars, all of which have  $\dot{P} \geq 10^{-16}$ . The probability of a chance clustering of five pulsars in 23 out of 62 is about 1%. Even if chance explains the clustering effect, it does not explain the anomalously low  $\dot{P}$  values.

The above results suggest that the barycentre of the Solar System is accelerated in a direction that is within or close to the coordinate values already given. If  $\dot{V}$  is the acceleration of the Solar System in a direction defined by coordinates  $\alpha_0$ ,  $\delta_0$ , and  $\Delta\dot{P}$  is the change in  $\dot{P}$  of a pulsar located at coordinates  $\alpha$ ,  $\delta$ , then

$$\frac{\Delta\dot{P}}{P} = -\frac{\dot{V}}{c} \{ \sin\delta\sin\delta_0 + \cos\delta\cos\delta_0\cos(\alpha - \alpha_0) \} \quad (2)$$

Evidently,  $-\Delta\dot{P} \sim 10^{-16}$  is sufficient to account for the low values of  $\dot{P}$  in Table 1, including correction for the Shklovsky effect, and because  $P \sim 1$  s, this corresponds to an acceleration  $\dot{V} \sim 10^{-6} \text{ cm s}^{-2}$ . This acceleration is very roughly in the direction of the galactic centre but is at least two orders of magnitude greater than the acceleration of the Sun in the gravitational field of the Galaxy. (More precise values of  $\dot{V}$ ,  $\alpha_0$ ,  $\delta_0$  cannot be found by averaging a weighted  $\dot{P}$  over the celestial sphere. This is because of a selection effect: the pulsars in the direction of the galactic centre include those of higher displacement from the galactic plane and therefore they have lower average  $\dot{P}$ .)

An intriguing explanation of the acceleration  $\dot{V}$  is that the Sun has an orbiting companion star. The acceleration is then

$$\dot{V} = GM_c/R_c^2 = 0.6 M/R^2 \text{ cm s}^{-2} \quad (3)$$

and is directed toward the companion star of mass  $M_c$  at distance  $R_c$ , where  $M (= M_c/M_\odot)$  is its mass measured in units of the Sun's

Table 1 Pulsars of  $\dot{P} < 10^{-16}$

Pulsar	$P$ (s)	$\dot{P}$ ( $10^{-16}$ )	Age ( $10^8$ yr)	Distance (pc)	Refs
1730-22	0.87	$0.0 \pm 0.2$	$> 10$	1,600	2,5
1813-26	0.59	$-3 \pm 3$	?	3,500	2
(1913+16)	0.06	$0.088 \pm 0.003$	$\sim 3$	6,200	12)*
1944+17	0.44	$0.244 \pm 0.005$	6	550	13
1952+29	0.43	$0.02 \pm 0.001$	70	265	14,15

\*Binary pulsar

mass and  $R$  its distance in AU. A companion star of mass  $M \sim 1$  at distance  $R \sim 10^3$  therefore produces an acceleration that is approximately equal to  $\dot{V} \sim 10^{-6} \text{ cm s}^{-2}$ . The circular orbital period of the hypothetical star is

$$P_c = R^{3/2}(1+M)^{-1/2} \text{ yr} \sim 10^4 \text{ yr} \quad (4)$$

and the corresponding proper motion is  $\sim 100$  arc s per yr (the annual parallax is  $\sim 200$  arc s).

Most nearby stars are members of systems that contain two or more stars<sup>18</sup>, and several stars have unseen companions detected astrometrically. The possibility of the Sun possessing a faint companion star has so far not been entirely eliminated<sup>19</sup>. A hypothetical companion star of, say, an apparent magnitude  $m_v = 10$  (much too faint to be seen by the naked eye), has a luminosity  $\sim 10^{-9}$  times that of the Sun. It is conceivable that the companion star is a crystallised white dwarf<sup>20,21</sup> that has cooled rather rapidly to a surface temperature of less than  $10^3$  K during the lifetime of the Solar System. A red (or even black) dwarf<sup>22</sup> of mass  $M \lesssim 0.1$ , that has failed to ignite hydrogen (but may be burning deuterium), has an exceedingly low luminosity and is therefore also a plausible candidate. If, however, the companion is a neutron star or black hole, produced by a supernova in the early history of the Solar System, then it is difficult to understand how our weakly bound binary system has survived such a violent event. It is more likely that a companion neutron star or black hole is in hyperbolic orbit, and the present close encounter is a transitory phenomenon lasting only several thousands of years. The companion star is therefore presumably either a faint white or red dwarf in closed orbit about the Sun, or a gas-accreting nearby neutron star or black hole in open orbit. It should not be difficult to detect low-luminosity objects of this kind having large annual parallax and proper motion.

Obviously, a star in closed orbit about the Sun must lie close to the ecliptic plane so as not to disturb the planets excessively out of their common orbital plane. According to current theories on star formation<sup>23</sup>, stars and their planets condense in interstellar gas clouds, and therefore planets probably revolve in the same orbital plane as the parent stars of a binary system. A companion star orbiting close to the ecliptic is not inconsistent with the distribution of pulsars of  $\dot{P} < 10^{-16}$  shown in Fig. 1.

A companion star in closed orbit would produce various long-term effects within the Solar System. For example, the apsidal period  $U$  (the period of precession of perihelion) of a planetary orbit of period  $P_p$  is<sup>24,25</sup>

$$U = \frac{4}{3}(1+M^{-1})P_c^2P_p^{-1} \quad (5)$$

and hence, with  $M \sim 1$ ,  $P_p \sim 1$  yr,  $P_c \sim 10^4$  yr, this equation yields  $U \sim 10^8$  yr, and the precession amounts 0.5 arc s per century (or 0.1 arc s per century in the case of Mercury). Short-term perturbations in the motions of Neptune and Pluto are significant, but as far as I can determine, are not unacceptably large.

Long-term changes in climate are conceivably attributable to periodicities in the Earth's orbit<sup>26,27</sup>, that could be enhanced by the presence of a companion star, particularly if its orbit has appreciable ellipticity. Furthermore, cometary motions will undoubtedly be affected. According to Oort's comet-cloud theory<sup>28</sup>, the comets extend out to a distance  $\sim 10^5$  AU, and the comet cloud



therefore envelops the proposed binary system of the Sun and its companion star. Oort has suggested that the nearby stars, at a few light years distance, perturb the comet cloud and as a result comets continually diffuse into orbits of comparatively small perihelia. The proposed binary system would obviously play an even more effective role in stirring the cometary orbits, and could also explain why the comet cloud, during the lifetime of the Solar System, has expanded to its present size.

Has the Sun a companion star? I find it hard to believe that a star so close can exist and yet remain undiscovered. On the other hand, pulsar observations of extraordinary precision imply that it might exist, and therefore a search for a companion star is perhaps worth undertaking.

I thank T. T. Arny, D. J. Helfand, W. C. Saslaw, and particularly J. H. Taylor, for helpful comments, and also to the Aspen Center for Astrophysics for hospitality. Helfand has recently measured  $\dot{P}$  for PSR2106+44 and finds that for this pulsar:  $\dot{P} = -1.36 (\pm 1.00) \times 10^{-17}$ . It is interesting that this pulsar is in the same region of the sky as the other pulsars of anomalously low  $\dot{P}$ . I also thank Dr P. van de Kamp for his comments, and for pointing out that the 'train whistle effect' has been known since 1901 as 'perspective secular change in the line of sight'<sup>29</sup>.

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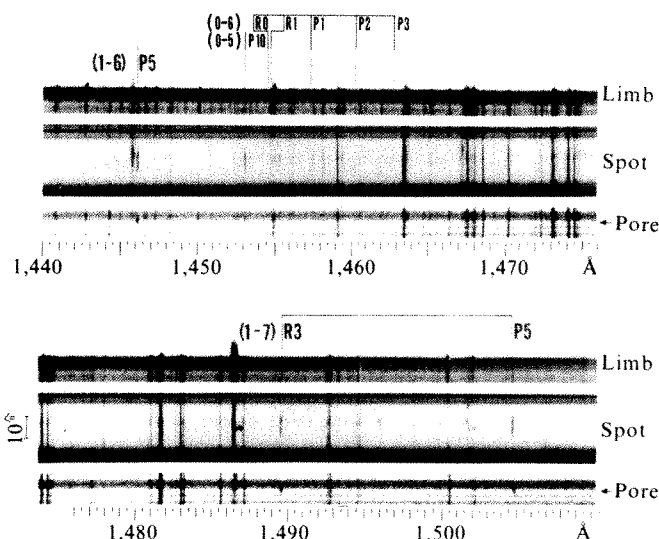
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- Gunn, J. E. & Ostriker, J. P. *Astrophys. J.* **160**, 979 (1970).
- Lyne, A. G., Ritchings, R. T. & Smith, F. G. *Mon. Not. R. astr. Soc.* **171**, 579 (1975).
- Harrison, E. R. & Tademaru, E. *Astrophys. J.* **201**, 447 (1975).
- Helfand, D. J. & Tademaru, E. *Nature* **267**, 130 (1977).
- Helfand, D. J. & Tademaru, E. *Astrophys. J.* **216**, 842 (1977).
- Taylor, J. H. & Manchester, R. N. *Astr. J.* **80**, 794 (1975).
- Terzian, Y. & Davidson, K. *Astrophys. Space Sci.* **44**, 479 (1976).
- Flowers, E. & Ruderman, M. A. *Astrophys. J.* **215**, 302 (1977).
- Macy, W. W. *Astrophys. J.* **190**, 153 (1974).
- Jones, P. B. *Nature* **262**, 120 (1976).
- Hulse, R. A. & Taylor, J. H. *Astrophys. J. Lett.* **195**, L51 (1975).
- Taylor, J. H., Hulse, R. A., Fowler, L. A., Gullahorn, G. E. & Rankin, J. M. *Astrophys. J. Lett.* **206**, L53 (1976).
- Richards, D. W., Rankin, J. M. & Zeissig, G. A. *Nature* **251**, 37 (1974).
- Gullahorn, G. E., Payne, R. R., Rankin, J. M. & Richards, D. W. *Astrophys. J. Lett.* **205**, L151 (1976).
- Mansfield, V. N. & Rankin, J. M. *Vistas in Astronomy* (in the press).
- Shklovsky, I. S. *Astrophys. Lett.* **8**, 101 (1970).
- Helfand, D. J., Taylor, J. H. & Manchester, R. N. *Astrophys. J. Lett.* **213**, L1 (1977).
- Van de Kamp, P. A. *Rev. astr. Astrophys.* **9**, 103 (1971).
- Van de Kamp, P. *Pub. astr. Soc. Pac.* **73**, 404 (1961).
- Van Horn, H. M. *IAU Symp. White Dwarfs* (Reidel, Dordrecht, 1970).
- Ostriker, J. P. A. *Rev. astr. Astrophys.* **9**, 353 (1971).
- Kumar, S. S. *Low-Luminosity Stars* 255 (Gordon and Breach, New York, 1969).
- Jastrow, R. & Cameron, A. G. W. (eds) *Origin of the Solar System* (Academic, New York, 1963).
- Slavenas, P. *Trans. astr. Obs. Yale Univ.* **6**, 35 (1927).
- Kopal, Z. *Close Binary Systems* 108 (Wiley, New York, 1959).
- Ward, W. R. *J. geophys. Res.* **79**, 3375 (1974).
- Hays, J. D., Imbrie, J. & Shackleton, N. J. *Science* **194**, 1121 (1976).
- Oort, J. H. *Bull. astr. Inst. Neth.* **11**, 259 (1951).
- van de Kamp, P. *Elements of Astromechanics* 128 (Freeman, San Francisco, 1964).

## Lines of H<sub>2</sub> in extreme-ultraviolet solar spectra

THE first detection of H<sub>2</sub> in the Sun, in extreme ultraviolet spectra, is reported here. The Naval Research Laboratory's High Resolution Telescope and Spectrograph (HRTS), flown in a rocket on 21 July 1975, was used for these observations. The slit extended from Sun centre to limb and crossed a sunspot at 07° S, 23° W in McMath region 13766. This stigmatic spectrograph, described by Bartoe and Brueckner<sup>1</sup>, covered 1,175–1,714 Å with 0.06 Å spectral and 1.5 arc s spatial resolution. A full list of observed lines is in preparation<sup>2</sup>. The sunspot spectrum contains some 120 emission lines which do not correspond to known lines listed in compilations<sup>3</sup> of transitions in atoms or ions, apart from random coincidences. More recent publications referred to in the NBS Bibliography<sup>4</sup> were also consulted. The identifications of the lines which are enhanced in the sunspot spectrum will be discussed elsewhere<sup>5</sup>.

A short stretch of a spectrum obtained with a 51-s exposure time is reproduced in Fig. 1. The top spectrum is of the solar limb,



**Fig. 1** A short section of the stigmatic ultraviolet solar spectrum showing several of the Lyman band lines in the 1,440–1,510 Å region. Each of the two sections covers three different areas on the solar surface. The solar limb spectrum is at the top, the spot spectrum in the centre and the 'pore' spectrum at the bottom.

the centre one is of the sunspot and the lower one is of a region of the quiet Sun which contains a small area ( $\sim 2$  arc s) in which some of the sunspot lines are greatly enhanced (a 'pore').

At least three distinct types of lines are present. Some are observed in the spot, pore and at the limb (for example, the line at 1,446.13 Å in Fig. 1); others are restricted to the spot and limb or to the pore and limb.

The continuum in both the sunspot umbra and penumbra is below the instrumental threshold for detection even in the longest exposure of 51 s. At 1,600 Å this corresponds to an upper limit of 3,950 K for the region of formation of the continuum, compared with the normal quiet Sun value of  $\sim 4,400$  K. From their visible region spectra sunspots are found to have photospheric temperatures of  $\sim 4,400$  K, and the temperature minimum above a spot is typically considered to be  $\sim 3,200$  K (ref. 6). The visible region spectra are therefore characterised by the presence of low excitation absorption lines and by hundreds of absorption lines of molecular origin which do not appear in the normal photospheric spectrum. In the ultraviolet the known emission lines of singly ionised species decrease in intensity over the spot and only neutral lines formed deeper in the chromosphere are enhanced. The origin of the new lines is therefore restricted to species with low ionisation potentials or dissociation energies.

Because of the high abundance of hydrogen and the expected low temperatures, the molecule H<sub>2</sub> (dissociation energy 4.55 eV), which has strong far ultraviolet bands, is an obvious candidate for any new lines in the sunspot. An early comparison with known laboratory spectra<sup>7</sup> of H<sub>2</sub>, however, indicated no general agreement in the character of the spectra. In a 100 Å section near 1,500 Å there are of the order of 100 vibrational lines in the Lyman bands, whereas the sunspot spectrum over the same wavelength range shows only a few lines. Moreover, even allowing for different excitation conditions between the Sun and laboratory, most of the lines expected to be strong are absent.

But it has now been found that at least 30 of the new lines are due to known transitions in the Lyman bands of H<sub>2</sub>, the key to the appearance of the spectrum lying in the excitation mechanism for the lines.

The Lyman bands arise between the ground state  $1^1\Sigma_g^+$  and the excited state  $1^1\Sigma_u^+$ . The lines of many vibrational bands have been tabulated by Herzberg and Howe<sup>8</sup>, and a useful *Atlas of Intense Lines* has been prepared by Schubert and Hudson (unpublished Aerospace Report, ATN-64(9233)-2).

The most striking aspect of the lines which correspond to H<sub>2</sub> is that 13 of them can be identified at P-branch ( $J=5$ ) and R-branch

( $J=3$ ) transitions in the  $v'=1$  to  $v''=n(2<n<9)$  Lyman bands; only one line in each branch is observed in each band, all originating from a common  $v'=1, J'=4$  upper level. Progressions with upper vibrational quantum number  $v'=constant$  were first studied by Wood and Hackett<sup>8,9</sup> and are usually due to fluorescence.

The source of the fluorescence is not hard to find, given the high intensity of the nearby H L $\alpha$  line. The energy difference between the  $v'=1, J'=4$  level in  $^1\Sigma_u^+$  and the  $v''=2, J''=5$  level in  $^1\Sigma_g^+$  is within  $26\text{ cm}^{-1}$  of the energy of H L $\alpha$ . The full-width at half maximum (FWHM) of H L $\alpha$  is  $\sim 1\text{ \AA}$ . So the H<sub>2</sub> molecules in the sunspot, or pore, can be excited by photons in the red wing of H L $\alpha$  as they travel down towards the photosphere.

The observed lines from  $v'=1$  are listed in Table 1, together with their intensities in the umbra, and their FWHM. The theoretical intensity ratio<sup>10</sup> of P5 to R3 lines is 10/8, and within the limits of experimental accuracy the observed line intensities agree with this. Along the progression the intensities follow closely the relative strengths of the bands as predicted by the transition probabilities of Allison and Dalgarno<sup>11</sup>. The observed line widths correspond to a 'thermal' temperature of  $7.7 \times 10^3\text{ K}$ , or with  $T_e \sim 4,400\text{ K}$ , a nonthermal velocity of  $\sim 5\text{ km s}^{-1}$ .

**Table 1** Lines observed in the  $v'=1$  to  $v''=n(2<n<9)$  Lyman bands of H<sub>2</sub>

Transition $v'v''$	$\lambda^*$ ( $\text{\AA}$ )	Intensity ( $\text{erg cm}^{-2}\text{s}^{-1}\text{st}^{-1}$ )	FWHM ( $\text{\AA}$ )	Comments
1-2 P5	(1,216.05)	—	—	Masked and excited by L $\alpha$
R3	1,202.45	—	—	Weak in umbra
1-3 P5	1,271.93	11	0.090	
R3	1,257.80	11	0.083	
1-4 P5	(1,329.14)	—	—	Masked by C(I) 1,329.10
R3	1,314.62	3	—	
1-5 P5	1,387.35	2	—	
R3	1,372.49	—	—	Weak in umbra
1-6 P5	1,446.13	9.6	0.093	
R3	1,431.01	7.4	0.088	
1-7 P5	1,504.79	14	0.093	
R3	1,489.57	12	0.094	
1-8 P5	1,562.41	12	0.114	
R3	1,547.35	14	0.101	
1-9 P5	1,617.93	—	—	Weak in umbra
R3	1,603.24	—	—	Weak in umbra

\*The wavelengths are from Herzberg and Howe<sup>7</sup>. Solar wavelengths agree with laboratory wavelengths within 0.02  $\text{\AA}$ .

The 1-0 and 1-1 bands are outside the wavelength range of the instrument, but an 'anti-Stokes' line is observed at 1,202.45  $\text{\AA}$ . Other fluorescence occurs, accounting for transitions in the 0-4, 0-5 and 0-6 bands. To produce these the  $v'=0, J'=1$  and 2 levels are excited from  $v''=2, J''=1$  by photons  $104\text{ cm}^{-1}$  and  $134\text{ cm}^{-1}$  out into the red wing of H L $\alpha$ . The  $J''=9$  level is also excited but not apparently by photons. But its energy is close to that of the  $v'=1, J'=4$  level discussed above and collisional processes may be indicated. This second group of lines is listed in Table 2. There are a further 20 or so weak lines of similar appearance which correspond by wavelength to H<sub>2</sub> transitions but which do not show such systematic behaviour.

The closest coincidence with H L $\alpha$ , the transition between  $v'=3, J'=1$  and  $v''=3, J''=0$ , does not produce observable lines. This is probably due to the combined effects of the self-reversal of H L $\alpha$ , a lower transition probability and a lower population of  $v''=3$ .

The remaining lines, which do not appear in the pore, may also have a molecular origin, but the singly ionised rare-earths are another possible source of low excitation lines.

The aspects of the data which relate to the understanding of the solar atmosphere will be discussed in the forthcoming paper<sup>5</sup>. But

**Table 2** Lines observed in the  $v'=0$  to  $v''=4,5,6$  Lyman bands of H<sub>2</sub>

Transition $v'v''$	$\lambda^*$ ( $\text{\AA}$ )	Intensity ( $\text{erg cm}^{-2}\text{s}^{-1}\text{st}^{-1}$ )	FWHM ( $\text{\AA}$ )	Comments
0-4 P1	(1,335.87)	—	—	Masked by C(II) 1,335.70
P2	1,338.57	7.2	0.083	
P3	1,342.26	—	—	Weak
P10	1,393.45	4.0	—	Blend?
R0	1,333.48	5.4	0.099	
R1	1,333.80	—	—	Weak
R8	1,363.58	5.1	0.092	
0-5 P1	1,396.22	—	—	Weak
P2	1,398.96	—	—	Weak
P3	(1,402.65)	—	—	Masked by Si(IV) 1,402.79
P10	1,453.02	7.7	0.105	
R0	(1,393.72)	—	—	Masked by Si(IV) 1,393.78
R1	(1,393.96)	—	—	Masked by Si(IV) 1,393.78
R8	1,422.55	1.8	0.085	
0-6 P1	1,457.43	—	—	Weak
P2	1,460.17	2.4	0.068	
P3	1,463.83	2.6	0.095	
P10	—	—	—	
R0	1,454.83	—	—	Weak
R1	1,454.97	3.2	0.091	b1 1455.00?
R8	—	—	—	

\*The wavelengths are from Herzberg and Howe. Solar wavelengths agree with laboratory wavelengths within 0.02  $\text{\AA}$ .

the importance of this H L $\alpha$ , H<sub>2</sub> fluorescence process extends beyond the solar atmosphere, since it will occur wherever strong L $\alpha$  radiation can penetrate a layer of temperature  $\sim 2,000\text{--}5,000\text{ K}$ . For example, one can immediately predict that these H<sub>2</sub> lines should be present in stars cooler than the Sun, particularly those of type early K, since the visible region spot 'special type' is K0. Such observations could be made with the forthcoming International Ultraviolet Explorer (IUE) Satellite, which includes the appropriate wavelength range. Further, Osterbrock<sup>12</sup> and Black and Dalgarno<sup>13</sup> have stressed the importance of the ultraviolet radiation field in the formation of the interstellar lines of H<sub>2</sub>. The penetration of L $\alpha$  from hot stars into nearby cooler media may play a particularly important role, although the low population of the  $v''=2$  levels will make fluorescence less effective than in stellar atmospheres.

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1. Bartoe, J.-D. F. & Brueckner, G. E. *J. opt. Soc. Am.* **65**, 13-21 (1975).
2. Sandlin, G. D., Bartoe, J.-D. F., Brueckner, G. E., Van Hoosier, M. E., *Astrophys. J. Suppl. Ser.* (to be submitted).
3. Kelly, R. L. & Palumbo, L. *J. N.R.L. Rep.* 7599 (1973).
4. Hagan, L. *NBS Spec. Publ.* 363, Suppl. 1 (1977).
5. Jordan, C., Brueckner, G. E., Bartoe, J.-D. F., Sandlin, G. D. & Van Hoosier, M. E., *Astrophys. J.* (to be submitted).
6. Allen, C. W. *Astrophysical Quantities* 3rd edn, 185 (Athlone, London, 1973).
7. Herzberg, G. & Howe, L. L. *Can. J. Phys.* **37**, 636-659 (1959).
8. Wood, R. W. *Phil. Mag.* **12**, 499-522 (1966).
9. Wood, R. W. & Hackett, F. E. *Astrophys. J.* **30**, 330-372 (1909).
10. Herzberg, G. *Spectra of Diatomic Molecules* (Van Nostrand, Princeton, 1950).
11. Allison, A. C. & Dalgarno, A. *Atomic Data* **1**, 289-304 (1970).
12. Osterbrock, D. E. *Astrophys. J.* **136**, 359-364 (1962).
13. Black, J. H. & Dalgarno, A. *Bull. Am. astr. Soc.* **6**, 444 (1974).

## Significance of peroxyntiric acid in atmospheric chemistry of nitrogen oxides

KNOWLEDGE of the life cycle and budget of nitrogen oxides in the atmosphere is important for several reasons. In the stratosphere, NO and NO<sub>2</sub> (NO<sub>x</sub>) play a dominant part in maintaining the structure and stability of the ozone distribution. In the troposphere, NO<sub>x</sub> is involved in the production and destruction of OH radicals which control the fluxes of many important species such as CH<sub>4</sub>, CO, H<sub>2</sub> and CH<sub>3</sub>Cl, to the stratosphere. In polluted urban areas, NO<sub>x</sub> chemistry provides the basic formation mechanism of photochemical air pollution. In all these areas of atmospheric chemistry, the interactions of NO<sub>x</sub> with HO<sub>x</sub> species (HO, HO<sub>2</sub> and so on) are of great significance, particularly the reactions of HO<sub>2</sub> with NO and of HO with NO<sub>2</sub>. A further HO<sub>x</sub>-NO<sub>x</sub> interaction is the proposed<sup>1</sup> formation of peroxyntiric acid in reactions (1) and (-1):

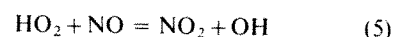
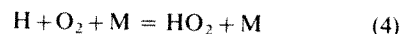
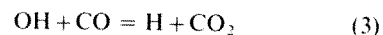
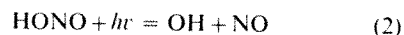


We present here a study of the extent to which reaction (1) and (-1) acts as a sink for HO<sub>x</sub> and NO<sub>x</sub> in the atmosphere.

Peroxyntiric acid was first reported to possess rather low stability at room temperature in the gas phase<sup>1</sup>, decomposing back to HO<sub>2</sub> and NO<sub>2</sub> with a lifetime of about 50 s. Subsequently other groups<sup>2,3</sup> have identified infrared absorptions of HO<sub>2</sub>NO<sub>2</sub>, reporting much longer lifetimes, about 500 s, with the decay being ascribed to heterogeneous removal at the vessel walls. These apparently diverging conclusions can be rationalised if HO<sub>2</sub>NO<sub>2</sub> is in thermal equilibrium with HO<sub>2</sub> and NO<sub>2</sub> as we have shown to be the case for its organic analogue PAN (peroxyacetyl nitrate) and the corresponding peroxy radical, CH<sub>3</sub>CO(O<sub>2</sub>) (ref. 4). When NO<sub>2</sub> is in large excess over NO, as in the infrared experiments, the main fate of the HO<sub>2</sub> radical is to recombine with NO<sub>2</sub> reforming HO<sub>2</sub>NO<sub>2</sub> again. Only when nitric oxide can effectively compete against NO<sub>2</sub> for HO<sub>2</sub>, will HO<sub>2</sub>NO<sub>2</sub> decay at its unperturbed unimolecular decay rate,  $k_{-1}$ .

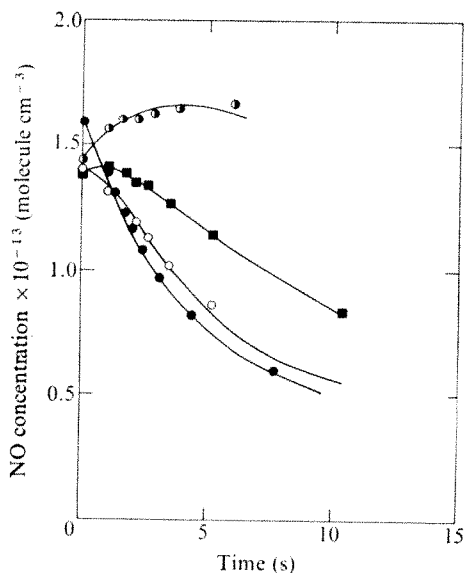
We have studied the temperature dependence of this competition for HO<sub>2</sub> radicals in reactions (1) and (5) by producing HO<sub>2</sub>

radicals from the photolysis of nitrous acid (HONO) in the presence of carbon monoxide (CO):

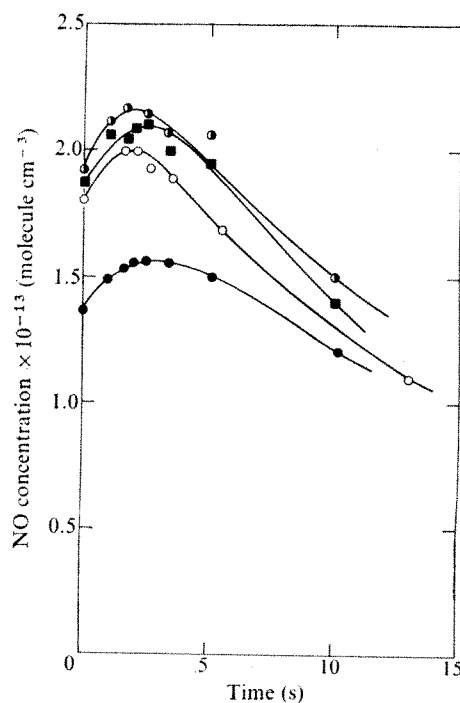


The photolysis was conducted in a flow system<sup>5</sup> with reaction times of 1–13 s. NO<sub>x</sub> was present at p.p.m. concentrations in synthetic air diluent at 1 atm pressure; NO, NO<sub>2</sub> and HONO were measured by chemiluminescence techniques<sup>6</sup>.

The observed time dependence of the nitric oxide concentration, [NO], reflects the balance between production by photolysis of HONO and NO<sub>2</sub> and by oxidation through the chain reaction sequence, (3) + (4) + (5) (see Figs 1 and 2). Figure 1 shows that [NO] usually declines, indicating the dominance of chain oxidation over photolytic production. But increasing the NO<sub>2</sub> concentration leads to decreased nitric oxide decay rates. The magnitude of the observed effect requires HO<sub>2</sub>NO<sub>2</sub> formation to be the explanation



**Fig. 1** NO concentration-time curves showing the effect of increasing NO<sub>2</sub> on the inhibition of NO oxidation in mixtures containing approximately  $2 \times 10^{14}$  and  $5 \times 10^{16}$  molecule cm<sup>-3</sup> of HONO and CO in 1 atm of air at 35 °C. Initial NO<sub>2</sub> concentrations, [NO<sub>2</sub>]<sub>0</sub> in molecule cm<sup>-3</sup> are: ●,  $1.78 \times 10^{13}$ ; ○,  $3.24 \times 10^{13}$ ; ■,  $5.90 \times 10^{13}$ ; ○,  $1.15 \times 10^{14}$ . Lines show computed curves used in estimating  $k_1/k_5$  and  $k_{-1}$ .



**Fig. 2** NO concentration-time curves showing the effect of temperature on the inhibition of NO oxidation by the addition of approximately  $1 \times 10^{14}$  molecule cm<sup>-3</sup> NO<sub>2</sub> to mixtures containing HONO and CO in 1 atm of air. Experimental points: ●, 40 °C; ■, 45 °C; ○, 55 °C. Lines show computed curves using  $k_1/k_5 = 0.22$  and  $k_{-1}$  values illustrated in Fig. 3.

rather than increased NO<sub>2</sub> photolysis. Figure 2 shows that the inhibiting effect of HO<sub>2</sub>NO<sub>2</sub> formation is only transitory. The initial increase in [NO] due to strong inhibition of the chain oxidation gives way to a steady decline at a rate almost independent of temperature. The induction period, representing the time taken for [NO] to reach its maximum, increases with decreasing temperature. This behaviour is exactly that expected for the formation of an intermediate which is thermally unstable with respect to active free radical chain carriers. Thus peroxyntiric acid is apparently a kinetically significant species in the HO<sub>x</sub>+NO<sub>x</sub> system at low temperatures.

Quantitative kinetic information was obtained by integrating the differential equations describing the reaction scheme, using the Harwell computer program FACSIMILE<sup>7</sup> and literature rate constants<sup>8</sup>. The values of  $k_1$  and  $k_{-1}$  were obtained by minimising

the sum of the squares of the deviations between observed and simulated [NO] against time curves. The experiments below 295 K gave consistent values of  $0.22 \pm 0.03$  for the rate constant ratio  $k_1/k_5$  in air at 1 atm total pressure. In these conditions the  $\text{HO}_2\text{NO}_2$  lifetime was longer than the residence time in the flow reactor so that redissociation was unimportant and the [NO] against time profile was determined primarily by the relative rates of reactions (1) and (5). This ratio  $k_1/k_5$  was then used at the higher temperatures to obtain the values of  $k_{-1}$  which are plotted in Arrhenius form in Fig. 3. A value of  $1.2 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$  (ref. 9) was adopted for  $k_5$  but neither  $k_{-1}$  or  $k_1/k_5$  were sensitive to the chosen value of  $k_5$  in the range  $10^{-11}$  to  $10^{-12}$ . The values of  $k_{-1}$  are represented by  $k_{-1} = 10^{16.1 \pm 1.6} \exp(-11,700 \pm 1,100/T) \text{ s}^{-1}$  for  $T$  in K at 1 atm pressure. The pre-exponential factor and activation energy,  $(97 \pm 9) \text{ kJ mol}^{-1}$ , are quite reasonable for the unimolecular decomposition of a weakly bound molecule into two radical fragments.

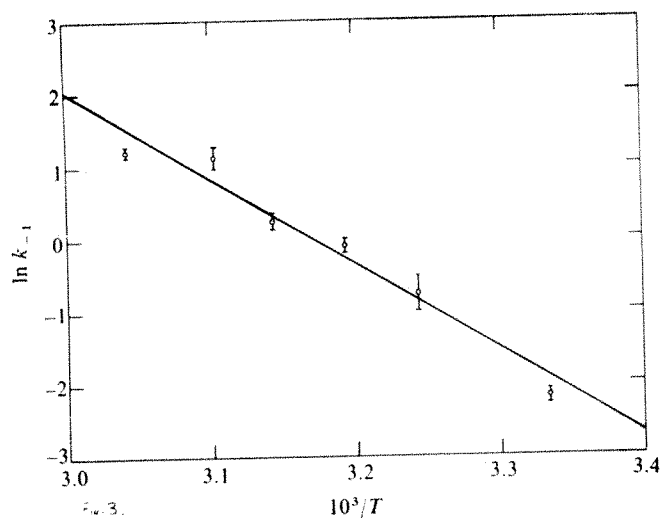


Fig. 3 Temperature dependence of the decomposition rate constant for  $\text{HO}_2\text{NO}_2$ ,  $k_{-1}$ : plot of  $\ln k_{-1}$  against  $10^3/T$  ( $\text{deg K}^{-1}$ ).

Decomposition lifetimes of  $\text{HO}_2\text{NO}_2$  calculated from the above expression for  $k_{-1}$  give some indication of the stability of  $\text{HO}_2\text{NO}_2$  at various altitudes in the atmosphere. In the coolest parts of the atmosphere near the tropopause, mean temperatures are about 220 K, corresponding to an  $\text{HO}_2\text{NO}_2$  lifetime of about 16 weeks (0.3 yr) with respect to thermal decomposition. As a result, the concentration distribution of  $\text{HO}_2\text{NO}_2$  in the upper troposphere and lower stratosphere will depend on the atmospheric circulation and dynamics as well as on the chemistry, provided that  $\text{HO}_2\text{NO}_2$  is not removed rapidly by photolysis and reactions with other stratospheric species. Calculations with a two-dimensional model indicate that as much as 30% of the total  $\text{NO}_x$  in the upper troposphere may become tied up as  $\text{HO}_2\text{NO}_2$ . This species may therefore play a similar role to  $\text{HNO}_3$  in providing a removal mechanism for stratospheric  $\text{NO}_x$ .

In the atmosphere near the Earth's surface  $\text{HO}_2\text{NO}_2$  is unstable with a decomposition lifetime of 9 s at 298 K.  $\text{HO}_2\text{NO}_2$  will therefore not provide a significant sink or store for  $\text{HO}_2$  or  $\text{NO}_2$ , merely existing at a low steady-state concentration in the sunlit atmosphere. Consequently  $\text{HO}_2\text{NO}_2$  is likely to have only a limited significance in photochemical air pollution and model calculations of photooxidant formation will not be seriously in error by omitting this species. This conclusion is in contrast to that of Levine *et al.*<sup>3</sup> who suggest that  $\text{HO}_2\text{NO}_2$  may be an important product of photochemical smog.

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1. Simonaitis, R. & Heicklen, J. *J. phys. Chem.* **80**, 1-7 (1976).
2. Niki, H., Maker, P. D., Savage, C. M. & Breitenbach, L. P. *Chem. Phys. Lett.* **45**, 564-566 (1977).
3. Levine, S. Z., Uselman, W. M., Chan, W. H., Calvert, J. G. & Shaw, J. H. *Chem. Phys. Lett.* **48**, 528-535 (1977).
4. Cox, R. A. & Rolley, M. J. *Environ. Sci. Technol.* **11**, 900-906 (1977).
5. Cox, R. A. *J. Photochem.* **3**, 175-188 (1974).
6. Atkins, D. H. F. & Cox, R. A. *A.E.R.E. Rep. R-7615* (Her Majesty's Stationery Office, London, 1973).
7. Curtis, A. R. & Kirby, C. R. *CSSD Rep. CSS 26; CSS 46* (Atomic Energy Research Establishment, Harwell, 1973).
8. Hampson, R. F. & Garvin, D. *NBS Technical Note 866* (US Department of Commerce, Washington, 1975).
9. Cox, R. A. & Derwent, R. G. *J. Photochem.* **4**, 139-153 (1975).

## Vertical profile of stratospheric ozone by lidar sounding from the ground

LIDAR measurements of stratospheric ozone by differential absorption (DIAL) technique were taken during July 1977 at the Haute-Provence Observatory (44N). A frequency doubled rhodamine 6G dye laser provided an output energy of 20-40 mJ in the wavelength range 300-310 nm. We report here preliminary measurements of stratospheric ozone in the altitude range 18-25 km. This experiment was carried out with the lidar facility previously used for the study of upper atmospheric metallic constituents at the mesopause level<sup>1</sup>.

The transmitting part of the lidar system consists of a flash-lamp pumped, frequency-doubled dye laser emitting in the wavelength range 300-310 nm. The experimental set-up is shown on Fig. 1 and its characteristics are summarised in Table 1. The dynamic range of this electronic system together with the close proximity of the emitting and receiving optical parts does not allow observations in a low altitude range (5-15 km); in order to avoid signal induced noise<sup>3</sup> or spurious signal from photomultiplier overloading, a rotating wheel protects the photomultiplier during the first 100  $\mu\text{s}$ . These constraints limit our experiments to an upper altitude range of 18-28 km. Observations have been conducted on two different nights (19-20 July, 20-21 July 1977) and more than  $5 \times 10^3$  laser shots have been registered and analysed.

The signal backscattered by Rayleigh diffusion in the altitude range  $z, z + \Delta z$  at a wavelength  $\lambda_1$  can be written as

$$N_R(\lambda_1, z) = N_L(\lambda_1) \frac{A}{z^2} \frac{d\sigma_r}{d\Omega}(\lambda_1) n_a(z) \Delta z \eta T^2(\lambda_1, z) \exp[-2\tau_{0.3}(\lambda_1, 0, z)] \quad (1)$$

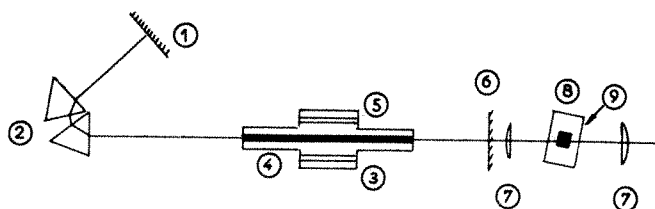
where  $N_L(\lambda_1)$  is the number of emitted photons,  $A$  the telescope area,  $\eta$  the optical efficiency of the lidar system,  $d\sigma_r/d\Omega(\lambda_1)$  the

Table 1 Characteristics of the lidar system

Transmitter		Receiver	
Energy per pulse	20-40 mJ	Telescope area	0.5 m <sup>2</sup>
Output wavelength	303.7-308 nm	Beamwidth	$5 \times 10^{-3}$ rad
Linewidth	0.15 nm	Linewidth	3 nm
Beam divergence	$< 8 \times 10^{-4}$ rad		
Pulse duration	4 $\mu\text{s}$		
Pulse repetition rate	0.25 Hz		

The receiver is a 0.8-m Coudé telescope. Two interference filters were used for these observations: they are centred respectively at 308.2 nm and 303.7 nm with a peak transmission of 30% and a width of 3 nm (FWHM). These wavelengths have been selected to obtain a ratio of 1.75 in the absorption cross-sections of ozone and are compatible with an observation in the stratospheric range. The Rayleigh backscattered signal is detected by a trialkali cathode photomultiplier (EMR 541 E) and its temporal analysis is made by a multichannel analyser working in a photon counting mode with a vertical resolution of 1.2 km.





**Fig. 1** The laser experimental set-up. 1, High reflectivity mirror; 2, wavelength selecting prisms; 3, laser head; 4, dye cell; 5, linear flash tubes; 6, output mirror ( $R = 40\%$ ); 7, cylindrical lens; 8, crystal housing (UG5 output window); 9, ADP crystal. The oscillator head is made of three 15-cm length bielliptical cylindrical reflectors, successively mounted with a  $60^\circ$  angle between their plane of symmetry to obtain a total pumping length of 45 cm. Six linear flash tubes (VQX6FD15) are then used in this optical arrangement. The fundamental line (600–620 nm) is generated using an active solution of rhodamine 6G ( $10^{-4}$  Ml $^{-1}$ ) in water with 4% Ammonix LO. With water as a solvent, the length of the laser cavity has been increased up to 2 m to reduce the beam divergence to less than  $8 \times 10^{-4}$  radian, leading to a better efficiency in the Second Harmonic Generation (SHG)<sup>2</sup>. Spectral narrowing of the emitted line to 0.15 nm is made using two quartz prisms working at their minimum deviation angle, which are also used to polarise the emitted beam. Rotation of the high reflectivity mirror leads to the tuning of the output wavelength which can be controlled using a grating spectrograph (dispersion 0.3 nm mm $^{-1}$ ). The output energy of 1 J which is usually obtained with an efficiency of 0.25% in a shot by shot experiment, has been reduced to half of this value for the atmospheric observations in order to increase the lifetime of the electrical components and to reduce the photolysis of the lasing solution. An ammonium dihydrogen phosphate crystal oriented for critical phase matching angle at the selected output wavelength is used to perform the SHG with an energy efficiency of 5% to 10% depending on the output power of the fundamental beam. Due to the presence of a 3 mm-thick UG5 window at the output edge of the crystal housing, only the ultraviolet light is emitted in order to avoid contamination effect of the backscattered signal by the fundamental visible beam.

Rayleigh backscattering cross-section,  $n_a(z)$  the total atmospheric density and  $T(\lambda_1, z)$  the atmospheric transmission between the ground and altitude  $z$  in the absence of ozone absorption.

$$\tau_{03}(\lambda_1, 0, z) = \int_0^z \sigma_{03}(\lambda_1) n_{03}(h) dh$$

is then the optical thickness of ozone at wavelength  $\lambda_1$  on a one-way path between the ground and altitude  $z$ . Its value is derived

from the experimentally measured  $N_R(\lambda_1, z)$  assuming that ozone is the main absorber at wavelength  $\lambda_1$  between  $z$  and  $z + dz$ . Interference effects with other minor constituents ( $\text{SO}_2$ ,  $\text{H}_2\text{O}$ , aerosols) are important in the troposphere<sup>4</sup> but their concentration are thought to be too low in the stratosphere range to produce any detectable attenuation. Absorption by molecular oxygen is also negligible at 300 nm where the cross-sections are of the order of  $10^{-26}$  cm $^2$  (ref. 5) which leads to an optical thickness of  $10^{-3}$  for a 1.2 km resolution range.

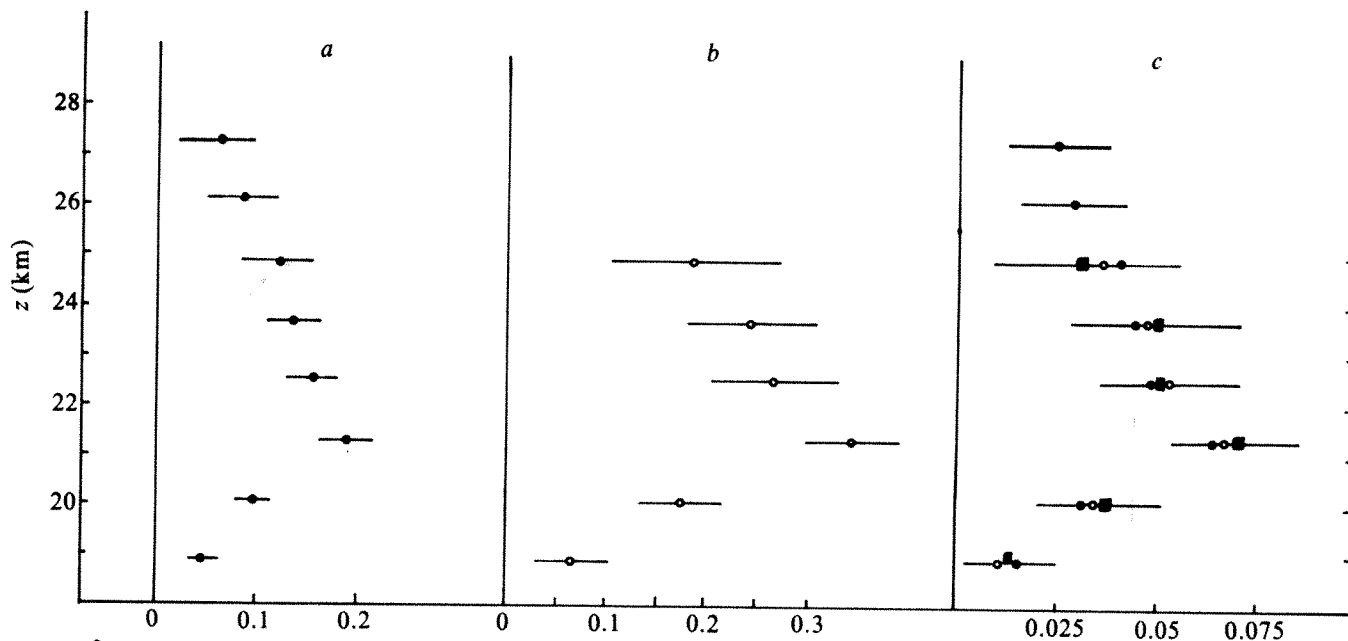
The effect of aerosols around 22 km (ref. 6) should also be considered assuming a maximum extinction coefficient of  $4 \times 10^{-8}$  cm $^{-1}$  at 300 nm and thus an optical thickness of  $5 \times 10^{-3}$  which is again one order of magnitude lower than the expected ozone absorption. These assumptions will be confirmed later by comparing signals obtained at two different wavelengths. The only contribution to the atmospheric transmission  $T(\lambda_1, z)$  is then the total molecular extinction by Rayleigh scattering which can be written as  $T(\lambda_1, z) = \exp[-\tau_a(\lambda_1, 0, z)]$  where  $\tau_a$  is the optical thickness defined as:

$$\tau_a(\lambda_1, 0, z) = \int_0^z \frac{d\sigma_r(\lambda_1) n_a(h)}{d\Omega} dh$$

From the ratio:

$$R(\lambda_1, z, z + \Delta z) = \frac{N_R(\lambda_1, z)}{N_R(\lambda_1, z + \Delta z)} \frac{(z + \Delta z)^2}{z^2} \frac{n_a(z)}{n_a(z + \Delta z)} \times \exp[-2\tau_a(\lambda_1, z, z + \Delta z)] \exp[-2\tau_{03}(\lambda_1, z, z + \Delta z)] \quad (2)$$

and using an atmospheric density model for midlatitude summer conditions<sup>7</sup> to derive the values of  $[n_a(z)/n_a(z + \Delta z)] \exp[-2\tau_a(\lambda_1, z, z + \Delta z)]$ , we can now deduce the optical thickness of the ozone layer between  $z$  and  $z + \Delta z$ . Figure 2a and b show these values of  $\tau_{03}$  between 18 km and 28 km with a resolution of 1.2 km, as measured at two different wavelengths ( $\lambda_1 = 308.2$  nm,  $\lambda_2 = 303.7$  nm). The deviation from a pure Rayleigh atmosphere shows up clearly. The integration time, 3 h during the night of 20 July, corresponds to  $3 \times 10^3$  laser shots at



**Fig. 2** a, Deviation of the differential optical thickness at  $\lambda_1 = 308$  nm from a pure Rayleigh atmosphere ( $\tau_{03} = 0$  corresponds to the situation of pure Rayleigh scattering). b, Deviation of the differential optical thickness at  $\lambda_2 = 303.7$  nm from a pure Rayleigh atmosphere. ( $\tau_{03} = 0$  corresponds to the situation of pure Rayleigh scattering). c, Comparison of  $\tau_{03}(\lambda_1) - \tau_{03}(\lambda_2)$  as measured from equation (3),  $\tau_{03}(\lambda_1)$  and  $\tau_{03}(\lambda_2)$  normalised to the same absorption cross-section ( $10^{-19}$  cm $^2$ ). ( $\tau/\sigma = 0$  corresponds to the situation of pure Rayleigh scattering.) (●,  $\tau/\sigma$  (308.2 nm); ○,  $\tau/\sigma$  (303.7 nm); ■,  $(\tau(\lambda_1) - \tau(\lambda_2))/(\sigma(\lambda_1) - \sigma(\lambda_2))$ ).

both wavelengths and leads to the computed error bars, taking into account the statistical error of the received signals.

If we consider the ratio

$$\mathcal{R} = \frac{R(\lambda_1, z, z + \Delta z)}{R(\lambda_2, z, z + \Delta z)} = \exp -2[\tau_{O_3}(\lambda_1) - \tau_{O_3}(\lambda_2)] \quad (3)$$

we then obtain a direct determination of the ozone concentration without reference to any model. The backscattering by atmospheric particles has the same effect at two wavelengths and thus is eliminated. We have plotted on Fig. 2c the values of  $(\tau_{O_3}(\lambda_1) - \tau_{O_3}(\lambda_2)) \times 10^{19}/(\sigma_{O_3}(\lambda_1) - \sigma_{O_3}(\lambda_2))$ , together with the related error bars as computed from the received signal, assuming the following values for the absorption cross-section<sup>5,8</sup>

$$\sigma_{O_3}(\lambda_1 = 308.2 \text{ nm}) = 3 \times 10^{-19} \text{ cm}^2$$

$$\sigma_{O_3}(\lambda_2 = 303.7 \text{ nm}) = 5.2 \times 10^{-19} \text{ cm}^2$$

These values are directly related to the ozone concentration and are compared with the ratios

$$\frac{\tau_{O_3}(\lambda_1) \times 10^{19}}{\sigma_{O_3}(\lambda_1)} \text{ and } \frac{\tau_{O_3}(\lambda_2) \times 10^{19}}{\sigma_{O_3}(\lambda_2)}$$

which lead to the same physical quantity. This shows good agreement between the two methods of data analysis confirming the former assumptions and the neutral density modelling.

The vertical ozone profiles obtained on these two nights are presented in Fig. 3 together as an example with the statistical error bar as derived for one night (20–21 July). The accuracy of the measurement is directly calculated from the backscattered signals using equation (3) except for the upper part of the profile ( $z > 25 \text{ km}$ ) where the absorption at 303.7 nm is too strong to

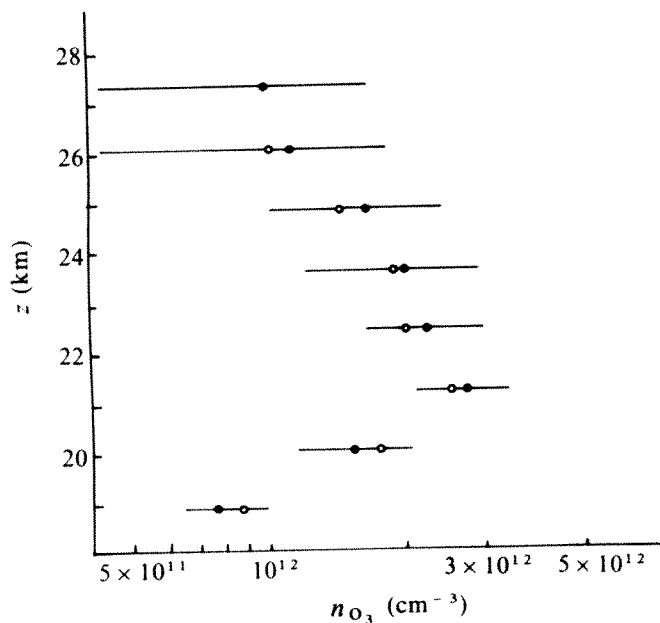


Fig. 3 Vertical ozone profiles (○, 19–20 July 1977; ●, 20–21 July 1977).

allow a precise determination of  $\mathcal{R}$  using this channel.  $n_{O_3}$  is then obtained only from the 308 nm channel assuming a constant value for the atmospheric density scaleheight, as calibrated in the lower altitude range by comparison of  $\tau_{O_3}(\lambda_1)$  and  $\tau_{O_3}(\lambda_2)$ . The accuracy of these preliminary measurements precludes any conclusion about a possible day to day variation of the ozone content. Its vertical repartition shows a maximum around 22 km with a concentration of  $3 \times 10^{12} \text{ cm}^{-3}$  which is within the range of measurements by other methods<sup>9</sup> and model computations<sup>10</sup>.

Further improvements of this DIAL technique to measure the ozone vertical profile will be done soon by extending the altitude range of the observations. Measurements of the backscattered signal in the analogical mode will allow the determination of  $n_{O_3}$  between 10–20 km whereas, in the lower troposphere, interference effects by other atmospheric minor constituents must be carefully considered. In a same way an increase of the output wavelength of the ultraviolet laser up to 320 nm will reduce the ozone absorption in the lower stratosphere and thus extend the altitude range of observations up to 40 km. Furthermore, the accuracy of the experiment could be improved by increasing the output energy of the ultraviolet laser up to a factor of 3 as already obtained in laboratory and its repetition rate (up to 2 Hz). This differential absorption lidar technique will then provide a very powerful tool for the determination of stratospheric ozone concentration from ground based stations.

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1. Mégie, G., Bos, F., Blamont, J. E. & Chanin, M. L. *Planet. Space Sci.* (in the press).
2. Hirth, A., Vollrath, K. & Allan, J. Y. *Opt. Com.* **20**, 347 (1977).
3. Pettifer, R. E. W. *J. atmos. terr. Phys.* **37**, 669 (1975).
4. Gibson, A. J. & Thomas, L. *Nature* **256**, 561 (1975).
5. Ackerman, M. *Mesospheric Model and Related Experiments* (ed. Fiocco, G.) 149 (Reidel, Dordrecht, 1970).
6. Castleman, A. W., Jr. *Physics and Chemistry of Upper Atmosphere* (ed. McCormac, B. M.) 143 (Reidel, Dordrecht, 1972).
7. *U.S. Standard Atmosphere suppl.* (1966).
8. Griggs, M. J. *Chem. Phys.* **49**, 857 (1968).
9. *The Natural Stratospheric CIAP Monograph 1* (Washington, D. C. 1974).
10. Krueger, A. J. & Minzner, R. A. *J. geophys. Res.* **81**, 4477 (1976).

## Photomineralisation rate of organic compounds adsorbed on particulate matter

ATTEMPTS to correlate the concentration of chemicals in the environment with their production figures have resulted in a large deficit, especially for organic chemicals such as DDT<sup>1</sup>. It has been assumed that analytical errors accounted for this deficit. Another explanation, however, considers the reactions of compounds in abiotic conditions. As far as atmospheric chemistry is concerned, such an explanation needs a thorough knowledge of the kinetics and mechanisms of all possible reactions of a compound, including its products and also consideration of the meteorological conditions. Only then would it be possible to describe the time dependence of concentrations of organic compounds in the atmosphere with any accuracy. To consider all the data required is time-consuming and expensive, because of the rather complicated mechanisms of conversion and decomposition. We have therefore devised simple, standardised tests.

In previous experiments we demonstrated that even persistent chemicals were decomposed to  $\text{CO}_2$  (and in the case of chlorinated compounds also to  $\text{HCl}$  and/or  $\text{Cl}_2$ ) as solids in an oxygen stream<sup>2,3</sup>. The results of this decomposition—the photomineralisation—are given in Table 1. Cyclodiene insecticides, such as aldrin and dieldrin, mineralise during irradiation with Pyrex-filtered ultraviolet light. Using short-wave ultraviolet light ( $\lambda < 290 \text{ nm}$ ), the amount of mineralisation products is significantly increased. DDT, DDE and pentachlorophenol behave in the same way as aldrin and dieldrin, whereas results from irradiating pentachlorobenzene, hexachlorobenzene and certain chlorinated biphenyls (PCBs) lead us to assume that these compounds are only mineralised, when exposed to short-wave ultraviolet light. Photomineralisation is, therefore, a simple test evaluating the behaviour of chemicals in atmospheric conditions. The relative mineralisation rate which is used to compare one substance with others can be

**Table 1** Mineralisation products after ultraviolet irradiation of certain chlorinated hydrocarbons as solids in an oxygen stream

Compound	Mineralisation products (mg)			
	Quartz (2 d)		Pyrex (6 d)	
	CO <sub>2</sub>	HCl	CO <sub>2</sub>	HCl
Aldrin				
Dieldrin	51-70	19-28	8-11	3-4
Photodieldrin				
Hexachlorobenzene				
Pentachlorobenzene				
2,4,5,2',4',5'-Hexachlorobiphenyl	46-53	19-26	†	†
2,5,2',5'-Tetrachlorobiphenyl				
Pentachlorophenol				
DDT				
DDE	*	*	10-15	2-8

About 80 mg of the respective compound was distributed on the inside wall of a 1-litre irradiation vessel and irradiated in an oxygen stream. The irradiation was carried out through quartz- and Pyrex-glass, using a Hg-high pressure lamp (HPK 125 W, Philips) as a light source.

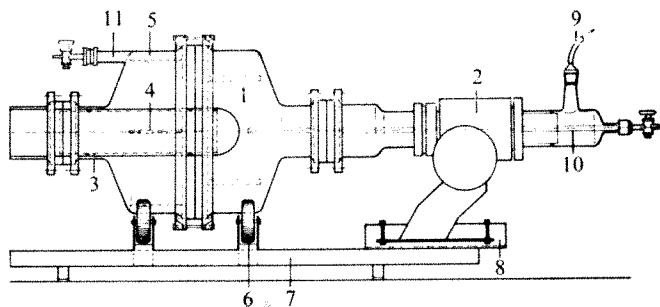
\*Not determined.

†Not detected.

easily determined by the mineralisation products formed, such as CO<sub>2</sub>, HCl, Cl<sub>2</sub>. But standardised and well controlled test conditions are necessary.

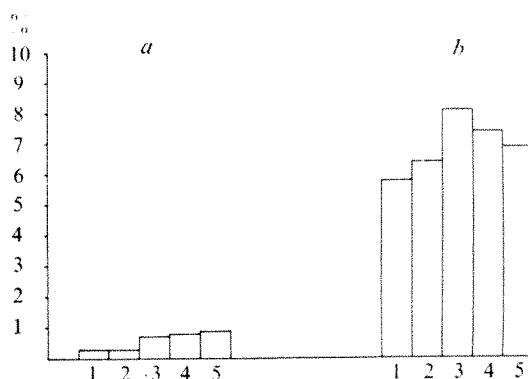
As a considerable fraction of the organic compounds in the atmosphere is adsorbed on particles, we use carrier materials in photomineralisation experiments and because of their readily standardisable properties, silica gel and aluminium oxide are good carriers. Using a suitable ratio of test substance to carrier material, a constant, approximately monomolecular dispersion on the surface can be assumed. The resulting substance-oxygen contact is more effective than that of solids and with the catalytic effects of the carrier material will increase the mineralisation rate. In addition the application of carrier materials was found to be particularly useful for comparing gaseous, liquid and solid exponents.

The photomineralisation of some chlorinated alkenes (1, 1-dichloroethylene, tetrachloroethylene, 2,3-dichloropropene, 1,1-dichloropropene and hexachlorobutadiene),



**Fig. 1** Section of the irradiation apparatus for photomineralisation of adsorbed substances. The cylindrical reactor drum (1) is on one end linked by means of flanged joints, able to transmit power, with a drive motor (2) whose control is infinitely variable. On the other end a closed-ended irradiation tube (3) which is welded with a glass coupling and attached to the reactor drum by a coupling, projects into the interior of the reactor. The dimensions of the irradiation tube are chosen so that different sources of light (4) and filters can be used. On the inner wall of the reactor drum blades (5), parallel to the longitudinal axis, are inserted, which occupy only a fraction of the diameter and are adjusted into staggered positions starting from the front end. The weight of the reactor drum is carried by four rollers (6), which are connected to a support (7) by a holding device. On the support the flexible suspension for the drive motor is arranged. The reactor drum can be charged either through tube (10) at the stationary part of the apparatus or through tube (11) by applying a vacuum to tube (9). By rotation of the reactor drum along its longitudinal axis the reactants (carrier material with adsorbed phase and gas phase) are completely tumbled and mixed so that a homogeneous irradiation is guaranteed.

freons (trichlorofluoromethane and dichlorodifluoromethane) and aromatic hydrocarbons (benzene, toluene, and *ortho*-, *meta*- and *para*-xylenes) in the adsorbed state was carried out in a special irradiation apparatus (Fig. 1)<sup>4</sup>. About 1.3 g of the test substance was applied to 800 g of silica gel using a stream of nitrogen gas. The amount of substance actually adsorbed was determined by gas chromatography and by elementary analysis. Silica gel (350 g) containing the test substance were introduced into the irradiation drum which was then evacuated twice to 100 mm Hg and flooded with pure oxygen (at a drum capacity of about 25 l, a large excess of oxygen is ensured in respect to a total mineralisation of the initial amount adsorbed). The ultraviolet irradiation of the adsorbed substances was carried out with a Hg-high pressure lamp (HPK 125 W, Philips) using filters of quartz or Pyrex glass for 3 or 6 d respectively. After the irradiation the mineralisation products, such as CO<sub>2</sub>, Cl<sub>2</sub> were driven off from the reactor drum by a carrier gas (N<sub>2</sub>) and trapped by passing the gas through two wash bottles connected in series, each containing 50 ml of 0.1 M NaOH solution. CO<sub>2</sub> was quantitatively determined by acidometric titration of CO<sub>3</sub><sup>2-</sup> and Cl<sub>2</sub> by iodometric titration of OCl<sup>-</sup>. The quantitative determination of mineralisation products which could not be eluted from the silica gel (such as HCl) was done by mixing silica gel with a NaOH solution (0.1 M) and subsequent potentiometric titration of Cl<sup>-</sup> with AgNO<sub>3</sub>. Table 2 and Fig. 2 show the results of the photomineralisation of the chlorinated alkenes, freons and aromatic hydrocarbons. In



**Fig. 2** CO<sub>2</sub>-formation of aromatic hydrocarbons adsorbed on silica gel by ultraviolet-irradiation. Percentages are given by moles CO<sub>2</sub> formed divided by moles CO<sub>2</sub> expected from a total mineralisation of the initial amount adsorbed. *a*, Pyrex, 6 d; *b*, quartz, 4 d. 1, Benzene; 2, toluene; 3, *o*-xylene; 4, *m*-xylene; 5, *p*-xylene.

all cases CO<sub>2</sub> was found; in the case of chlorinated compounds HCl and/or Cl<sub>2</sub> were also found.

The photomineralisation of chlorinated alkenes is the most effective as shown in Table 2. The quantity of the formed mineralisation products is not noticeably influenced by using quartz- or Pyrex-filtered ultraviolet light. The increased amount of products in the Pyrex experiments are due to the extended irradiation times. This indicates a possible photomineralisation with ultraviolet light predominating in the troposphere ( $\lambda > 290$  nm). Even the freons which are well known as highly persistent compounds are degraded to CO<sub>2</sub> and HCl by using Pyrex-filtered light. But here the degradation with short-wave ultraviolet light is slightly favoured.

In contrast to the chlorinated alkenes and freons, the aromatic compounds in the solid state and adsorbed on silica gel show a higher persistence with respect to a photomineralisation. While irradiating through Pyrex glass only low mineralisation rates were found. Using quartz-filtered

**Table 2** Photomineralisation of chlorinated alkenes and freons

	Quartz (3 d)		Pyrex (6 d)	
$\text{CCl}_2 = \text{C} - \text{C} = \text{CCl}_2$	*	§	†	§
$\begin{array}{c} \text{CH}_3 - \text{CH} = \text{CCl}_2 \\   \quad   \\ \text{H}_2\text{C} - \text{C} = \text{CH}_2 \\   \quad   \\ \text{Cl} \quad \text{Cl} \end{array}$	†	‡	†	§
$\begin{array}{c} \text{CH}_3 - \text{CH} = \text{CCl}_2 \\   \quad   \\ \text{H}_2\text{C} - \text{C} = \text{CH}_2 \\   \quad   \\ \text{Cl} \quad \text{Cl} \end{array}$	*	‡	†	§
$\text{Cl}_2\text{C} = \text{CCl}_2$	*	§	†	§
$\text{H}_2\text{C} = \text{CCl}_2$	*	‡	†	§
$\text{CCl}_3\text{F}$	*	§	*	‡
$\text{CCl}_2\text{F}_2$	*	‡	*	‡

Percentages are given by the amounts of  $\text{CO}_2/\text{Cl}$  actually formed divided by the amounts of  $\text{CO}_2/\text{Cl}$  expected from a total mineralisation of the initial quantity adsorbed.

\*10–50% HCl- and/or  $\text{Cl}_2$ -formation.

†50–90% HCl- and/or  $\text{Cl}_2$ -formation.

‡10–50%  $\text{CO}_2$ -formation.

§50–90%  $\text{CO}_2$ -formation.

light, however, a significant increase could be determined (Table 1 and Fig. 2).

An even greater distinction within one class of compounds is possible: by comparing benzene with toluene and the xylenes (Fig. 2) a tendency to higher  $\text{CO}_2$ -formation was found. Moreover, the three xylenes showed differences due to the steric position of their methyl groups. These results are supported by the determination of the remaining amounts of aromatic hydrocarbons after irradiation.

From these examples a characteristic behaviour of certain compounds as well as classes of substances becomes evident, which might help in evaluating organic chemicals with regard to their degradation in environmental conditions.

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1. Woodwell, G. M., Craig, P. P. & Johnson, H. A. *Science* **174**, 1102–1106 (1971).
2. Gäb, S., Parlar, H., Nitz, S., Hustert, K. & Korte, F. *Chemosphere* **3**, 183–186 (1974).
3. Gäb, S., Nitz, S., Parlar, H. & Korte, F. *Chemosphere* **4**, 251–256 (1975).
4. *Deutsche Patentanmeldung* P 27 04 942.9.

## Formation of Fe-Ni-Si planetary cores

THE planets may have accreted inhomogeneously materials accumulating on the proto-planets in the sequence in which they condense at high temperatures (1,000–2,000 K) out of the solar nebula<sup>1</sup>. The first solids to condense from the Earth, however, are the Ca and Al oxides, silicates and titanates<sup>2,3</sup> and not Fe or Fe-Ni alloy, which is supposed to form the Earth's core. Anderson and Hanks<sup>4</sup> consider that such a nucleus of oxides enriched in radioactive elements could provide a mechanism for heating and melting the surrounding solid Fe layer which accretes later. Ringwood<sup>5</sup> considers this model of core formation unsatisfactory since it may produce a layer of oxide rich material around the core for which we have no geophysical evidence. For Fe, or an alloy of Fe with other

elements, to form as the first equilibrium solid, the pressure in the solar nebula at 1,800 K must be several atmospheres<sup>3,6</sup>. But, the conditions for the formation of a liquid Fe or a liquid Fe-rich alloy have not been determined. If a liquid alloy formed as the first condensate, it would solve many problems in the current models of formation of planetary cores<sup>5</sup>. The pressure required to form a liquid alloy is likely to be an order of magnitude lower than the pressure for the formation of solid Fe because metal liquid solutions of Fe, Ni and Si have a lower free energy of solution than that of an ideal solution. In particular the Fe-liquid and Si-liquid, and Ni-liquid and Si-liquid form binary solutions with a low free energy which stabilises the liquid solutions at pressures lower than that of the solid. According to Podolak and Cameron<sup>6</sup> the models of Jupiter have an enhanced O-H ratio of ~25–30. They consider that pressure required to form chondrules will be lowered several orders of magnitude if the O/H ratio is increased. We report here an investigation into the equilibrium condensation of the solar nebular gas at 1,900 K at varying total pressures and varying abundance of hydrogen ( $A_H$ ).

The system consisted of 10 elements H, O, Al, Mg, Fe, Ni, C, Si, S, Ca in solar abundance<sup>7</sup> and over 70 gaseous and condensed compounds for which thermochemical data are available in sources listed by previous workers (such as Grossman<sup>8</sup>). The condensed compounds included forsterite and a number of liquid solutions of Fe, Ni and Si. These three elements were selected because they are liquids at 1,900 K. The vapour pressures of all other elements are low at 1,900 K and therefore none dissolves in the melt in any significant concentration. None of the oxides, titanates and other silicates condense at 1,900 K.

The free energies of the formation of liquid solutions were calculated using the expression

$$G = RT \sum X_i \ln X_i \gamma_i$$

Where  $G$  is the free energy of formation of the solution,  $X_i$  the mole fraction,  $\gamma_i$  the activity coefficient,  $R$  the gas constant and  $T$  the absolute temperature. For a ternary solution,  $\gamma_i$  is given by<sup>8</sup>:

$$\ln \gamma_i = X^2 [E_{ij} + 2 X_i (E_{ji} - E_{ij})] + X^2_k [E_{ik} + 2 X_i (E_{ki} - E_{ik})] + X_{jk} [0.5 (E_{ji} + E_{ij} + E_{ki} + E_{ik} - E_{jk}) + X_i (E_{ji} - E_{ij} + E_{ki} - E_{ik}) + (X_j - X_k) (E_{jk} - E_{kj}) - (1 - 2X_i) C]$$

Where the  $E$  values are the binary interaction coefficients and  $C$  is the ternary constant. Here we neglect the ternary constant. The data on binary coefficients are  $E_{\text{Fe-Si}} = -5.77$ ,  $E_{\text{Si-Fe}} = -7.41$ ,  $E_{\text{Ni-Si}} = -6.75$ ,  $E_{\text{Si-Ni}} = -19.43$ ,  $E_{\text{Fe-Ni}} = -0.67$  and  $E_{\text{Ni-Fe}} = -0.67$ . These coefficients have been calculated from the thermochemical data given by Fruehan<sup>9</sup> (Fe-Si), by Kubachewski *et al.*<sup>10</sup> (Ni-Si) and by Elliot *et al.*<sup>11</sup> (Fe-Ni).

The results of the calculations are shown in Fig. 1 and Table 1. Following the practice of Herndon and Suess<sup>12</sup>, in Fig. 1, we used the hydrogen depletion factor (here defined as  $D_H = \log A_H$  in solar gas/ $A_H$  chosen to vary the ratio; no other element is depleted). Under most conditions of pressure, and varying  $A_H$  the liquid that forms does not differ appreciably in composition from  $\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$ . At normal hydrogen abundance, the liquid does not form until the pressure exceeds 1 atm. With decreasing  $A_H$  ( $D_H = 1$ ) the minimum pressure at which liquid forms is between 0.1 and 0.2 atm. The amount of liquid increases rapidly with increasing pressure and by 1 atm most of the Fe and Ni have condensed along with forsterite ( $\text{Mg}_2\text{SiO}_4$ ). A significant amount of Fe and Ni (~60%) condense as liquid solution without any admixture with forsterite between 0.1 and 0.3 atm. Therefore if such conditions of pressure and hydrogen depletion could exist in the nebula over a length of time an alloy core with an outer layer of alloy and forsterite could form.

With a further decrease in  $A_H$  ( $D_H = 2$ ) the minimum pressure for liquid formation lies between 0.01 and 0.05 atm. This liquid, however, does not contain any Si. The concentration of



Table 1 Condensed species forming at 1,900 K

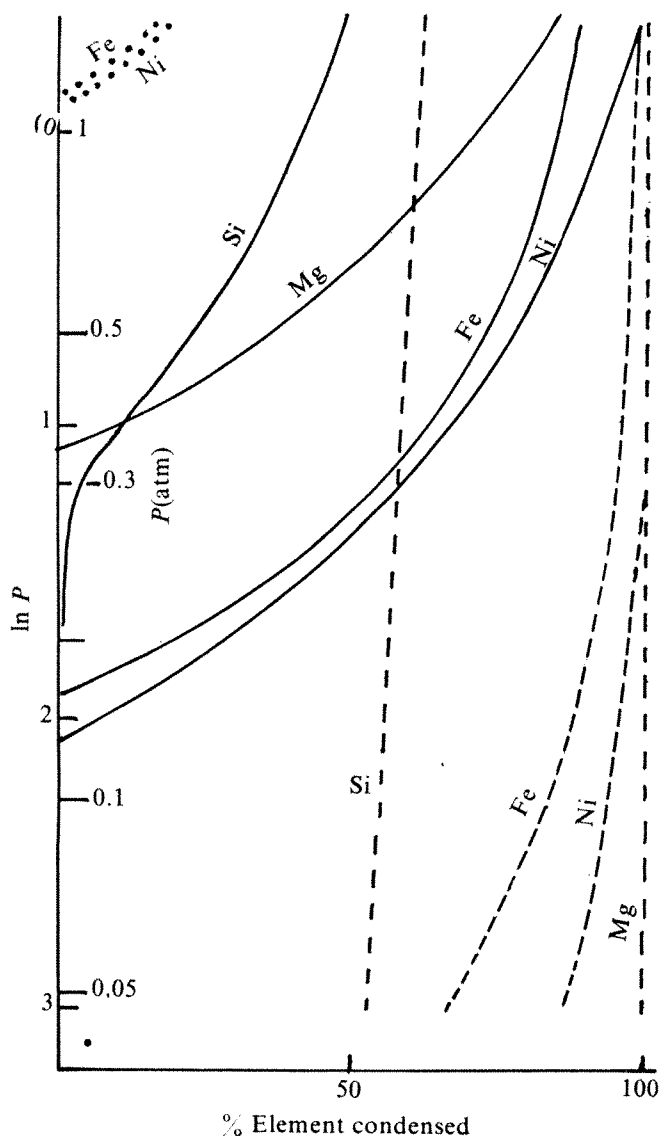
Pressure (atm)	$A_H = 2.6 \times 10^{10}$		$A_H = 2.6 \times 10^9$		$A_H = 2.6 \times 10^8$	
	Liquid	Solid	Liquid	Solid	Liquid	Solid
1.5	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$	None	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$	$\text{Mg}_2\text{SiO}_4$	$\text{Fe}_{0.906}\text{Ni}_{0.047}\text{Si}_{0.047}$	$\text{Mg}_2\text{SiO}_4$
1.0	None	None	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$	$\text{Mg}_2\text{SiO}_4$	$\text{Fe}_{0.903}\text{Ni}_{0.048}\text{Si}_{0.047}$	$\text{Mg}_2\text{SiO}_4$
0.5	None	None	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$	$\text{Mg}_2\text{SiO}_4$	$\text{Fe}_{0.903}\text{Ni}_{0.048}\text{Si}_{0.048}$	$\text{Mg}_2\text{SiO}_4$
0.4	None	None	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$	$\text{Mg}_2\text{SiO}_4$	$\text{Fe}_{0.902}\text{Ni}_{0.048}\text{Si}_{0.049}$	$\text{Mg}_2\text{SiO}_4$
0.3	None	None	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$	None	$\text{Fe}_{0.92}\text{Ni}_{0.05}\text{Si}_{0.03}$	$\text{Mg}_2\text{SiO}_4$
0.2	None	None	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.05}$	None	$\text{Fe}_{0.948}\text{Ni}_{0.052}$	$\text{Mg}_2\text{SiO}_4$
0.1	None	None	$\text{Fe}_{0.9}\text{Ni}_{0.05}\text{Si}_{0.04}$	None	$\text{Fe}_{0.945}\text{Ni}_{0.055}$	$\text{Mg}_2\text{SiO}_4$
0.05	None	None	None	None	$\text{Fe}_{0.938}\text{Ni}_{0.062}$	$\text{Mg}_2\text{SiO}_4$
0.01	None	None	None	None	None	$\text{Mg}_2\text{SiO}_4$

$A_H$ , hydrogen abundance. The compositions of the liquid solutions are approximate.

Si in liquid increases with pressure. At such a hydrogen abundance, Mg is nearly 100% condensed as forsterite at all pressure considered in this study.

Our conclusions are: (1) A core of liquid alloy could form from a gas of solar composition only at relatively high pressures of the order of 1 to 2 atm. (2) This pressure could be lowered by

Fig. 1 Percentage of elements condensed based on the results of phase equilibrium calculations. Fe, Ni and Si condense as liquid solution, Mg and Si as  $\text{Mg}_2\text{SiO}_4$ .  $D_H$  values as 0 (dotted line), 1 (solid line) and 2 (dashed line) represent hydrogen abundances of  $2.6 \times 10^{10}$ ,  $2.6 \times 10^9$  and  $2.6 \times 10^8$  respectively.



about a factor of 10 if hydrogen is depleted by a similar factor. (3) A greater hydrogen depletion ( $D_H = 1$ ) results in stabilising forsterite down to 0.01 atm (and lower). Significant amount of liquid ( $\text{Fe}_{0.94}\text{Ni}_{0.06}$ ) forms only at 0.5 atm which could be regarded as the lowest pressure for the formation of the liquid. (4) Silicon may be the light element component of the alloy only if pressures are higher than 0.2–0.3 atm. In the pressure range considered (0.2 to 1.5 atm) the concentration of silicon in the liquid solution varies between 3 and 5%. If the O/H conditions can be deduced from other mineral–chemical information, an appreciable concentration of Si in the metal phase could indicate formation at high nebular pressure.

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1. Turekian, K. K. & Clark, S. P., Jr *Earth planet. Sci. Lett.* **6**, 346 (1969).
2. Larimer, J. W. & Anders, E. *Geochim. cosmochim. Acta* **31**, 1239 (1967).
3. Grossman, L. *Geochim. cosmochim. Acta* **36**, 597 (1972).
4. Anderson, D. L. & Hanks, T. C. *Nature* **237**, 387 (1972).
5. Ringwood, A. E. *Composition and petrology of the earth's mantle* (McGraw-Hill, New York, 1975).
6. Podolak, M. & Cameron, A. G. W. *Icarus* **23**, 326 (1974).
7. Cameron, A. G. W. in *Origin and Distribution of the Elements* (ed. Ahrens, L. H.) 125 (Pergamon, Oxford, 1968).
8. King, M. B. *Phase Equilibrium in Mixtures* (Pergamon, Oxford, 1969).
9. Fruehan, R. J. *Met. Trans.* **1**, 865 (1970).
10. Kubachewski, O., Evans, E. L. I. & Alcock, C. B. *Metallurgical Thermochemistry* (Pergamon, Oxford, 1967).
11. Elliott, J. F., Gleiser, M. & Ramakrishna, V. *Thermochemistry for Steel-making* **2** (Addison-Wesley, Reading, Massachusetts, 1963).
12. Herndon, J. M. & Suess, H. E. *Geochim. cosmochim. Acta* **41**, 233 (1977).

## Angle of subduction

PLATE tectonics is well established as an empirical description for modification of the Earth's outermost solid layer, although the dynamics of the plates are poorly understood. One view is that the plates are surface manifestations of the deep mantle convection which must inevitably occur if there are deep-seated energy sources<sup>1</sup>. Mantle convection, however, may not be an efficient driver of plate motions<sup>2</sup>, and the negative buoyancy of subducting slabs may be more important<sup>2, 3</sup>. We consider here a simple fluid dynamical model which illustrates some of the general principles of subduction dynamics, and suggest an explanation for the observed subduction angle (the angle between the subducting slab and the Earth's surface). The model depends on the concept of a 'critical' negative buoyancy that plate material must attain before it can undergo steady-state subduction.

Observations indicate that the subduction angle  $\theta_s$  is seldom near  $0^\circ$  or  $90^\circ$  but is typically an intermediate value, say  $50^\circ$  (refs 4, 5). This does not seem to be explainable by trench migration (or the relative motion of plates) alone<sup>5</sup>. Jischke<sup>6</sup> explained the angle by proposing that the subducting slab adheres to the overriding continent, so that its angle of descent is dictated by the continental geometry, but this is only satisfactory for near surface behaviour. A detailed force-balance or energy-balance model might correctly predict  $\theta_s$ , but only by a fortuitous choice of poorly known parameters such as the average mantle viscosity  $\mu$ . In the energy minimisation calculation by Tullis<sup>8</sup>, for example, there is only a narrow range of  $\mu$  for which the predicted  $\theta_s$  is not near  $0^\circ$  or  $90^\circ$ . We propose, instead, that  $\theta_s$  is essentially independent of the poorly known mantle viscosity and plate rheology, and is determined by the requirement that the torques on the slab are balanced for the lowest possible negative buoyancy. The highly simplified model that we consider predicts  $\theta_s \sim 63^\circ$  for a non-migrating trench. Slab rigidity is fundamental to our model, but since the forces acting on the slab are a function of depth, deformation and even fracture of the slab may eventually occur and play a role in mantle seismic mechanisms<sup>7</sup>. Torque balance, however, with an appropriately chosen slab length, is a suitable first approximation for estimating  $\theta_s$ .

For simplicity, we assume that the mantle has a uniform viscosity and is convectively neutral, so that the only motion within it is that caused by the plates. We consider a two-dimensional model in which the trench and upper (non-subducting) plate are at rest (see Fig. 1). The subducting slab is assumed to be rigid and moving at a steady speed  $U$ . For the purpose of calculating the induced mantle flow only, it is also assumed to be infinitely long and infinitesimally thin. This is

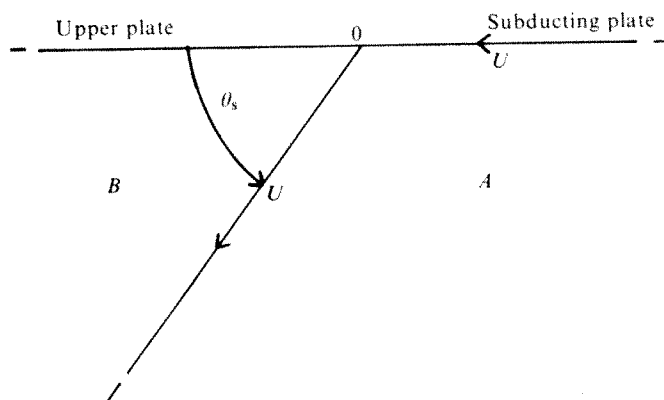


Fig. 1 Two-dimensional model for a subduction zone (see ref. 7). Material is transported from the ridge (to the right of the diagram) and undergoes subduction at 0. The upper plate is at rest.

the model considered by McKenzie<sup>8</sup>, whose results we use here. He gives the hydrodynamic contribution to the pressure in regions A and B of Fig. 1 are  $P_A(\theta)$ , and  $P_B(\theta)$  respectively, where

$$P_A(\theta) = \frac{2\mu U [\sin \theta - \sin(\theta - \theta_s)]}{r[(\pi - \theta_s) + \sin \theta_s]}$$

$$P_B(\theta) = \frac{-2\mu U [\sin \theta \sin \theta_s + \theta_s \sin(\theta_s - \theta)]}{r[\theta_s^2 - \sin^2 \theta_s]} \quad (1)$$

$\theta$  is the polar coordinate measured anticlockwise from the upper plate, and  $r$  is the radial coordinate measured from 0. The lubrication resulting from shear heating<sup>8</sup> and the diapiric upwellings which are responsible for behind-arc spreading<sup>9</sup> both modify these pressure estimates at small  $r$ , but this does not greatly affect the torque balance that we consider.

Since  $P_A(\theta_s) > 0 > P_B(\theta_s)$  for all  $\theta_s$ , the hydrodynamic pressure difference across the slab always acts to reduce  $\theta_s$ . The torque about 0 due to this suction is  $T_H$  per unit trench length, where

$$T_H = \int_0^L [P_A(\theta_s) - P_B(\theta_s)] r dr$$

$$= 2\mu UL \left[ \frac{\sin \theta_s}{(\pi - \theta_s) + \sin \theta_s} + \frac{\sin^2 \theta_s}{\theta_s^2 - \sin^2 \theta_s} \right] \quad (2)$$

and  $L$  is the length of the subducting slab (assumed independent of  $\theta_s$ ). We assume that  $T_H$  is balanced by the gravitational torque  $T_G$  caused by the negative buoyancy of the slab. If  $\Delta\rho(r)$  is the density contrast between slab and mantle, and  $h$  is the slab thickness, then

$$T_G = \int_0^L \Delta\rho(r) g h r \cos \theta_s dr$$

$$= \frac{1}{2} b L^2 \cos \theta_s$$

$$b = g h \int_0^1 \Delta\rho(x) dx \quad (3)$$

where  $x = r^2/L^2$ ,  $g$  is the gravitational acceleration, and  $b$  is the mean negative buoyancy per unit slab area. The inclusion of  $h$  in our definition of  $b$  emphasises that subduction is aided either by increasing the density contrast or by thickening the plate. We neglect the torque required to maintain the bend in the subducting plate. Although this cannot be justified *a priori*, it should be noted that whereas the work done at the trench may be large, it does not follow that the corresponding torque on the subducting slab is necessarily large.

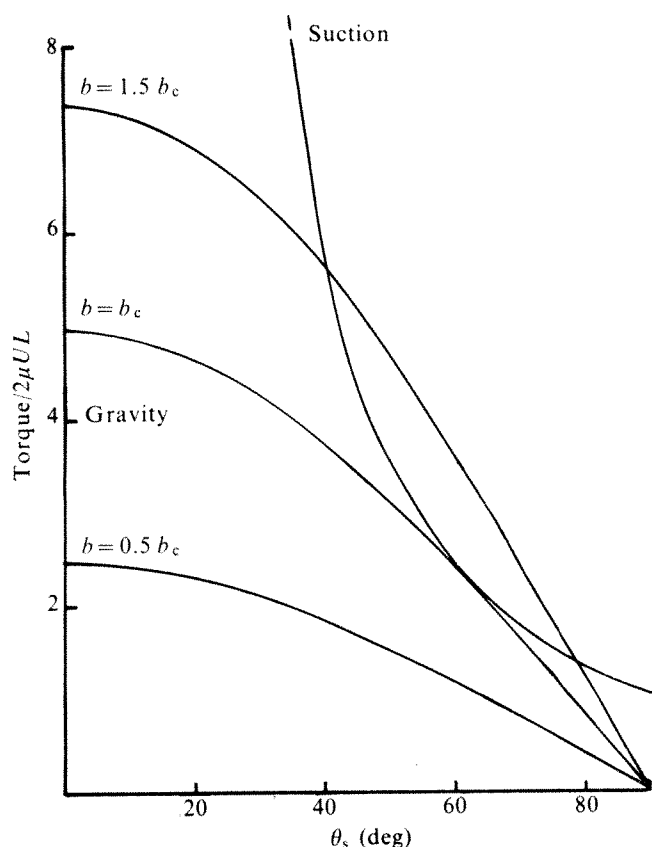
The graphical solution of  $T_H = T_G$  is shown in Fig. 2. Since  $T_H > 0$  for  $0 \leq \theta_s \leq \pi$  it follows that, for a fixed  $U$ , there is no solution for  $b$  sufficiently small. A solution becomes possible at a critical negative buoyancy  $b_c$  and a critical subduction angle  $\theta_c$  when the curves for  $T_H$  and  $T_G$  touch. The critical solution is

$$\theta_c \approx 63^\circ, b_c \approx 20\mu U/L \quad (4)$$

For  $b > b_c$ , two solutions are possible. The larger solution for  $\theta_s$  is stable since any small perturbation from it clearly leads to a restoring torque. The smaller solution for  $\theta_s$  is unstable, since any small deviation from it is amplified. The simple model predicts that  $\theta_s < 63^\circ$  is never stable. Subduction at these angles may be possible because of the finite torque required to bend the slab or the hydrodynamic pressure on the leading edge of the slab<sup>2</sup>.

This calculation provides no information on the onset of subduction since it concerns a steady state for which a well-developed subduction slab already exists. A model by Davies<sup>10</sup> may partly explain the initial subduction angle. We also do not consider the force balance along the direction of subduction which determines  $U$ . This balance is affected not only by the negative buoyancy and hydrodynamic effects<sup>2</sup>, but also by viscous drag on the subducting plate and ridge pushing (gravitational sliding)<sup>11</sup>. It is likely that  $U$  is not strongly dependent on  $\theta_s$ , but must itself exceed a critical value that is determined by the requirement that shear heating produce adequate lubrication below the plate between ridge and trench<sup>12, 13</sup>.

The following speculative but plausible picture emerges: after subduction is initiated, the trench migrates until a steady-state is reached where newly subducting material has the critical negative buoyancy  $b_c$  and subducts at an angle  $\theta_c$ .



**Fig. 2** The torques (in units of  $2\mu UL$ ) as a function of subduction angle. The three cosine curves labelled by values of  $b$  are for  $T_G$ . The remaining curve is for  $T_H$ . There is no solution for  $T_H = T_G$  if  $b < b_c$ , and a solution becomes possible at  $b = b_c$  when the curves just touch.

The negative buoyancy results from the thickening and cooling of the plate as the material proceeds from ridge to trench. The observed scatter of actual values for  $\theta_s$  results from the inevitable interaction between several ridge and trench systems, and the inability to achieve steady-state. The following plausible values satisfy equation (4) for  $b_c$ :  $\Delta\rho \sim 0.1 \text{ g cm}^{-3}$ ,  $h \sim 100 \text{ km}$ ,  $U \sim 10 \text{ cm yr}^{-1}$ ,  $L \sim 1,000 \text{ km}$ ,  $\mu \sim 2 \times 10^{22} \text{ P}$ . The size of the plate depends on the time taken for plate material to achieve critical negative buoyancy and, therefore, depends on the geothermal heat flux. This dependence may help to explain why plate tectonics was apparently so different in the Precambrian<sup>14</sup>.

The model we present here is oversimplified and incomplete, but it illustrates how the general physical principles of subduction might be identified. The choice of model is best made by comparing different tectonic systems, and data on the plate tectonics for the Earth at a different epoch or for other planets is essential for future progress.

After this work had been completed, the very closely related calculations by Tovish *et al.*<sup>15</sup> were brought to our attention.

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1. Turcotte, D. L. & Oxburgh, E. R. *An. Rev. Fluid Mech.* **4**, 33–68 (1972).
2. Richter, F. M. *Tectonophysics* **38**, 61–88 (1977).
3. Forsyth, D. & Uyeda, S. *Geophys. J. R. astr. Soc.* **43**, 163–200 (1975).
4. Luyendyk, B. P. *Geol. Soc. Am. Bull.* **81**, 3411–3416 (1970).
5. Tullis, T. E. Preprint (1977).
6. Jischke, M. C. *J. Geophys. Res.* **80**, 4809–4813 (1975).
7. Isaacs, B. & Molnar, P. *Nature* **228**, 1121–1124 (1969).
8. McKenzie, D. P. *Geophys. J. R. astr. Soc.* **18**, 1–32 (1969).
9. Oxburgh, E. R. & Parmentier, E. M. *J. Geol. Soc., Lond.* **133**, 343–355 (1977).

10. Davies, G. F. *Geophys. J. R. astr. Soc.* **49**, 557–563 (1977).
11. Hales, A. L. *Earth planet Sci. Lett.* **6**, 31–34 (1969).
12. Froidevaux, C. & Schubert, G. *J. Geophys. Res.* **80**, 2553–2564 (1975).
13. Melosh, H. J. *Tectonophysics* **35**, 363–390 (1976).
14. McElhinny, M. W. & McWilliams, M. O. *Tectonophysics* **40**, 139–159 (1977).
15. Tovish, A., Luyendyk, B. & Schubert, G. *Trans. Am. Geophys. Un. EOS* **57**, 1002 (1976).

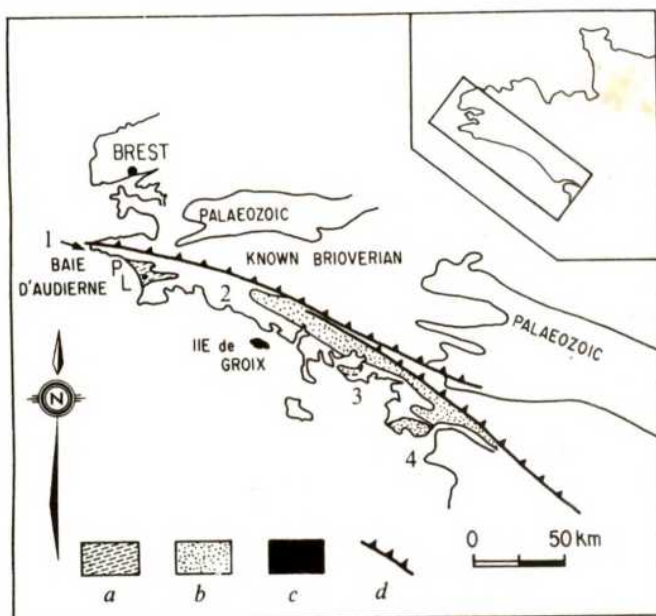
## Age and tectonic implications of the Baie d'Audierne basic-ultrabasic complex

MAJOR deformational and metamorphic events in the south-armoric metamorphic belt of south Brittany have been considered to be either Cadomian and Variscan<sup>1</sup> or Ordo-Silurian (Ligerian) and Variscan<sup>2</sup> in age. Geological interest in southern Brittany is, at least in part, focused upon the blueschists<sup>3</sup> and the basic-ultrabasic complexes of the area. The largest of these basic-ultrabasic complexes is that of the Baie d'Audierne group (Fig. 1). Although lacking suitable material for direct radiometric or stratigraphical dating, several indirect attempts have been made to date this group, using field data. These are briefly outlined here. It is proposed that the deformational and metamorphic history of this complex is entirely Variscan in age. The implications of this conclusion for the recently proposed plate-tectonic model of Cogné<sup>2</sup> are discussed.

The south-armoric metamorphic belt comprises a suite of non-fossiliferous, pre-Stephanian sediments, commonly ascribed to the late Proterozoic Brioverian<sup>4</sup>, which have been folded, metamorphosed and intruded by basic and ultrabasic rocks, granites and granite gneisses<sup>5,6</sup>. In the Baie d'Audierne region (Fig. 1), two divisions within the metamorphic rocks, an upper and a lower suite separated by an unconformity have been recognised<sup>7–9</sup>. It is thought that the lower suite was folded and metamorphosed in the amphibolite facies (kyanite grade) during the late Proterozoic Cadomian orogeny<sup>8</sup> before the deposition of the upper suite. The upper suite commenced with the deposition of a basal sedimentary series, the Languidou gneiss. Ordovician to Carboniferous sediments lie offshore of the Baie d'Audierne; their relationships to the upper suite is, however, uncertain<sup>9</sup>. In the Baie d'Audierne, the upper suite is cut by the Pors Poulhan granite gneiss (Fig. 1) which has yielded a whole rock Rb/Sr isochron at  $334 \pm 8 \text{ Myr}$  ( $\lambda^{87}\text{Rb} = 1.47 \cdot 10^{-11} \text{ yr}^{-1}$ ). This radiometric date has been interpreted as the magmatic cooling age of the granite. Subsequently, the whole sequence, upper and lower suites and the intruded Pors Poulhan granite, was folded by upright folds on east-west axes and metamorphosed in the albite-amphibolite facies during the Variscan orogeny<sup>8</sup>. Late Variscan granites intrude all the metamorphic rocks and are probably associated with other south-armoric granites emplaced into rocks of Upper and Lower Palaeozoic age<sup>11</sup>.

During recently completed fieldwork in the area where Cogné and Peucat defined the upper and lower Brioverian, in the Baie d'Audierne region (Fig. 1), I was unable to recognise any unconformity. The upper and lower suites throughout the area show a common structural and metamorphic history. In addition, the Languidou gneiss, interpreted by Cogné<sup>7</sup> as a metaconglomerate and used by Peucat<sup>8</sup> to support the postulated unconformity, is thought to be an intrusive granite containing partially assimilated xenoliths of country rock. The supposed 'pebbles' are deformed feldspar porphyroblasts and quartz-feldspar segregations. The Languidou gneiss shows the same pre-tectonic relationship to the surrounding metasediments as the Pors Poulhan gneiss. This pre-tectonic relationship also applies to an association of gabbro and peridotite (now amphibolite, garnet-pyroxenite and serpentinite, ascribed by Peucat to his lower suite) which intrude the metasediments. Following the intrusion of these ultrabasic to granitic rocks, the entire complex (the Baie d'Audierne group<sup>6</sup>) was isoclinally folded and metamorphosed in the amphibolite facies, up to sillimanite grade, with the development of an axial planar schistosity. This early schistosity was deformed by rare isoclinal folds and





**Fig. 1** Present-day distribution of the Baie d'Audierne group (a), south Brittany migmatites, (b) the Ile de Groix blueschists (c) and the spatial relationship to the Brioverian and Palaeozoic sediments north of the South Armorican Shear Zone (d); the blueschists may originally have been more widely separated from the mainland migmatites as they are bounded north and south by faults, possibly thrusts<sup>24</sup>. L, Languidou; P, Pors-Poulhan. 1, Cap Sizun gneisses; 2, Port Manech gneiss; 3, Morbihan; 4, Basse Loire (After Cogné<sup>1</sup>).

these in turn are refolded by upright folds on east-west axes, with a variably developed associated crenulation cleavage. The complex then underwent a mimetic annealing recrystallisation which did not produce any major retrogression of the earlier mineral assemblages. A subsequent retrogression in the greenschist facies is associated with the emplacement of the latest Variscan granites. A fuller account of the Baie d'Audierne area is given elsewhere<sup>6</sup>.

Similarly, the structural discordance representing the lateral extension of the unconformity within the metasediments of the metamorphic belt south of the South American Shear Zone has been observed neither in Morbihan<sup>12</sup> nor Basse Loire<sup>13</sup> (Fig. 1). From the above model, all the structural and metamorphic events affecting the rocks of the Baie d'Audierne group occurred between the emplacement of the Pors Poulhan gneiss and that of the late Variscan granites. If we accept the  $334 \pm 8$  Myr age given by the Pors Poulhan gneiss as an emplacement age, then the folding and metamorphism in the Baie d'Audierne area must have occurred entirely within the Upper Palaeozoic and forms part of the Variscan orogeny.

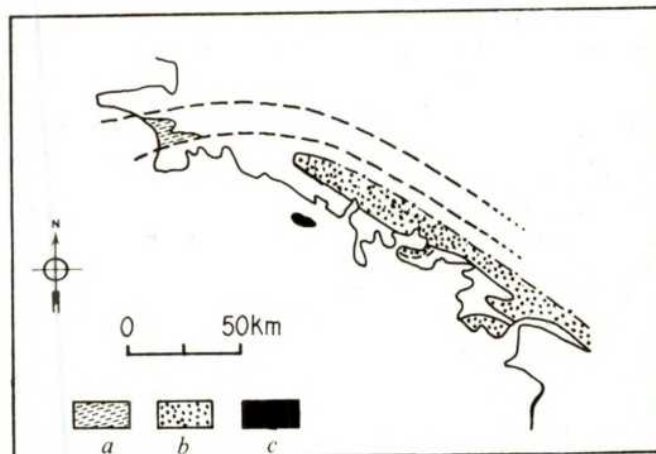
To the east, south east of the Baie d'Audierne, a suite of migmatites and anatectic granites outcrops (Fig. 1), within which the anatectic granite represents the culmination of migmatization<sup>1</sup>. To the south of the migmatites, metamorphic rocks of the blueschist (glaucofanite-lawsonite) facies outcrop on the Ile de Groix<sup>3,14,15</sup>. The anatectic granites have yielded a whole rock Rb/Sr isochron at  $363 \pm 12$  ( $\lambda^{87}\text{Rb} = 1.47 \times 10^{-11} \text{ yr}^{-1}$ ) (ref. 16). A premigmatization orthogneiss (Roguedas) has yielded a Rb/Sr whole rock isochron age at 460 Myr ( $\lambda^{87}\text{Rb} = 1.42 \cdot 10^{-11} \text{ yr}^{-1}$ ) (ref. 17), so fixing the migmatization between 460 Myr and 363 Myr. The diapiric rise of the anatectic granites is syn-second phase deformation in the surrounding migmatites<sup>18</sup>. This, in conjunction with the radiometric data, suggests the presence of a polyphase history of pre-Variscan<sup>2</sup>, high temperature metamorphism and deformation to the east whose early events and climax are older than the proposed post-334 Myr orogenic activity in the Baie d'Audierne to the west.

The radiometric ages for the migmatite suite are broadly similar to those for the time range of blueschist metamorphism

on the Ile de Groix (420–370 Myr) (ref. 17) as inferred from Rb/Sr mineral and whole rock data ( $\lambda^{87}\text{Rb} = 1.42 \cdot 10^{-11} \text{ yr}^{-1}$ ). A K/Ar 335 Myr mineral age has been suggested for this blueschist metamorphism<sup>19</sup>, which may, however, represent a later thermal event. In view of the spatial association of the above mentioned radiometric ages, rock types and metamorphic facies, it is possible to place the rocks of the Baie d'Audierne within the context of recently proposed tectonic models for south Brittany<sup>2,20</sup>. Such a tectonic context is necessarily confined to the south of the South Armorican Shear Zone due to the uncertainty concerning the movement history on this major tectonic line (P. Jegouzo, personal communication) and, therefore, the questionable validity of direct correlation between the south american metamorphic belt and central or north Brittany, the British Isles and so on.

The dominant strike trend in the Baie d'Audierne region is  $070^\circ$  (ref. 6); that of the migmatite belt to the east-south east is  $110-120^\circ$  (ref. 1). A gently curved arc can be envisaged, truncated by the late Variscan granites and mylonites of the South Armorican Shear Zone. Before the emplacement of these granites and the subsequent shear zone movement, the Baie d'Audierne group, which extends westwards to the edge of the submarine continental shelf<sup>9</sup>, would have extended to the east-north east, around the northern flank of the migmatite belt (Fig. 2). Whether or not the migmatite belt extended south of the present outcrop of the Baie d'Audierne group cannot be ascertained, as this position is occupied by a large, late Variscan granite (Pont l'Abbé). It is with this pre-late Variscan granite configuration in mind that the tectonic significance of the Baie d'Audierne group is discussed.

At, or even before, ~420 Myr ago, blueschist metamorphism was taking place to the south of a high temperature-medium

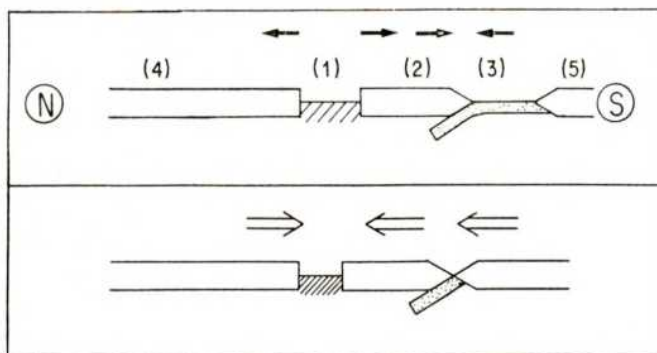


**Fig. 2** Proposed configuration of rocks of Baie d'Audierne (a), Migmatite Belt (b) and Ile de Groix blueschist (c) types pre-emplacement of the late Variscan granites. The true location and lateral extent of (c) cannot be ascertained<sup>24</sup>. Dashed lines indicate suggested boundaries.

pressure belt within which anatexis eventually occurred<sup>2</sup>. Relict areas of older granitic material (Port Manech and Cap Sizun gneisses) within the western part of the high temperature zone<sup>1,6,17</sup> suggest that the migmatite belt lay on, or immediately to the south of, continental crust (Fig. 3). The metamorphic belt therefore comprised an Andean type margin with a northward dipping subduction zone<sup>2,20</sup>.

Metamorphism and deformation of this margin during the Ordo-Silurian (Ligerian) orogeny<sup>3</sup> was related to subduction and the eventual arrival of northward moving continental material (Fig. 3), now lying immediately south of the blueschists (micaschists, granites and undifferentiated Palaeozoic sediments of the South Brittany continental shelf<sup>2,21</sup>). In the Baie d'Audierne area, sometime between the initiation of subduction (~420 Myr) and 334 Myr ago, rifting occurred





**Fig. 3** Non-scaled diagram of postulated plate boundary relationships during the Palaeozoic, representing an hypothetical approximately north-south cross section across Fig. 2: 1, Baie d'Audierne type basic and ultrabasic rocks; 2, south Brittany migmatites; 3, Ile de Groix blueschists associated with oceanic crust; 4, northern continental block; 5, southern continental block. Post-420 Myr/pre-334 Myr plate motions (solid arrows) involve northward subduction at the southern margin of the migmatitic island arc (2), behind-arc extension (1) and northward movement of the southern continental block. Post-334 Myr, the behind-arc rift closes (open arrows), as the island arc + southern continent (2+5) collide with the northern continental block (4). (Adapted after Cogné<sup>2</sup>).

within the continental plate just to the north of the migmatite belt (Fig. 3). Into this rift, mantle derived gabbro and peridotite were intruded, representing the initial stages in the development of a marginal basin behind an island arc<sup>22</sup>, now deeply eroded and represented by the migmatite belt. (A mid-ocean ridge origin for these basic and ultrabasic rocks has also been suggested<sup>8,23</sup>.)

At or post ~334 Myr ago, the migmatitic island arc, then in contact with the southern continental block (Fig. 3), was pushed northwards against the northern continental block, folding the behind arc rift zone and producing the Variscan deformation and metamorphism seen in the Baie d'Audierne. This continent plus island arc-continent collision corresponds to Cogné's intercontinental collision of the same age<sup>2</sup>. In Cogné's model, however, the 'suture' representing this collision is marked by the South Armorican Shear Zone to the north (Fig. 1).

The south-armoric metamorphic belt was situated therefore, at an active Andean type continental margin about 420 Myr ago, which after passing through a phase of back-arc rifting, progressed to a collision with a southern continent plus island arc and the northern continent, post 334 Myr ago.

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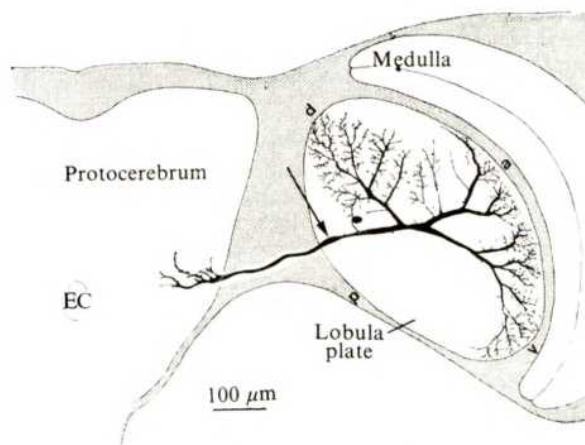
Received 4 May; accepted 19 September 1977.

1. Cogné, J. *Mem. Serv. Carte Géol. Fr.* 370 (1960).
2. Cogné, J. *Coll. Int. C.N.R.S. Géol. Himalaya* (in the press).
3. Cogné, J., Jeannette, D. & Ruhland, M. *Bull. Serv. Carte Géol. Als. Lorraine* 41-95 (1966).
4. Cogné, J. *Bull. Géol. Soc. Fr.* 4, 413-430 (1962).
5. Cogné, J. in *Géologie de la France* (ed. Debelmas, J.) 105-161 (Doin, Paris, 1974).
6. Hanmer, S. K. thesis, Univ. London (1977).
7. Cogné, J. *C.r. Acad. Sci. Paris* 261, 3849-52 (1965).
8. Peucat, J. J. thesis, Univ. Rennes (1973).
9. Lefort, J. P. & Peucat, J. J. *C.r. Acad. Sci. Paris* 279, 635-637 (1974).
10. Cogné, J. & Peucat, J. J. *C.r. Acad. Sci. Paris* 277, 2601-04, 1973.
11. Vidal, Ph. *Bull. géol. Soc. Fr.* 15, 239-245 (1973).
12. Audren, Cl. thesis, Univ. Rennes (1971).
13. Hassenforder, B. thesis, Univ. Strasbourg (1970).
14. Makanjua, Z. A. & Howie, R. A. *Contr. Mineral. Petrol.* 35, 83-118 (1972).
15. Carpenter, M. S. N. & Civetta, L. *Nature* 262, 276-277 (1976).
16. Vidal, Ph. thesis, Univ. Rennes (1976).
17. Peucat, J. J. & Cogné, J. *Nature* 268, 131-2 (1977).
18. Audren, Cl. & Le Metour, J. *Bull. géol. Soc. Fr.* 18, 1041-49 (1976).
19. Carpenter, M. S. N. thesis, Univ. Oxford (1976).
20. Nicholas, A. *Nature* 236, 221-223 (1972).
21. Lefort, J. P. thesis, Univ. Rennes (1976).
22. Oxburgh, E. R. *Proc. geol. Ass.* 85, 299-357 (1974).
23. Peucat, J. J. *Reun. Ann. Sci. Terre* 313 (1974).
24. Audren, Cl. & Lefort, J. P. *Bull. géol. Soc. Fr.* (in the press).

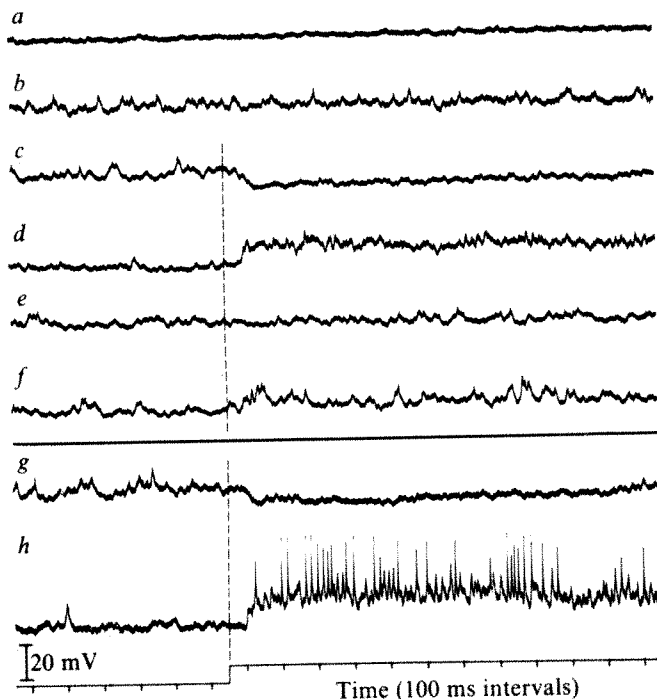
## Spike responses of 'non-spiking' visual interneurone

OUR understanding of information processing in nerve nets has been modified by the concept of graded signal transmission<sup>1</sup>. Descriptions of non-spiking interneurons in insects<sup>2-8</sup>, and the demonstration of graded synaptic transmission<sup>3</sup> have contributed to this development. In the fly visual nervous system, second and higher order interneurons are known, which apparently do not produce action potentials<sup>6-8</sup>. We show here that at least eight individually identifiable movement-sensitive cells, which have the characteristic properties of non-spiking interneurons<sup>2</sup> will generate spikes with imposed hyperpolarisation. Their graded mode of operation is due to maintained refractoriness. This applies selectively to neurones, which belong to either of two anatomically, and physiologically distinct classes. Other cell types in the same preparation generate spikes spontaneously.

The third visual neuropil of flies contains two conspicuous sets of directionally-selective movement-sensitive neurones<sup>9,10</sup>—an array of 10 'vertical cells' (VS 1-VS 10) which respond to vertical movements in the ipsilateral visual field, and an array of three 'horizontal cells' (HS 1-HS 3) which respond to horizontal rotary movements in the visual field of the two eyes<sup>7,8</sup>. Figure 2a-f shows some of the characteristic responses to visual stimuli of the neurone VS 1 depicted in Fig. 1. Its membrane potential in the dark is about -41 mV, modulated by sparse synaptic activity (Fig. 2a). Illumination of the receptive field causes a small depolarisation and a marked increase in 'noise' (Fig. 2b). When a striped pattern moves upwards in the receptive field, the cell is hyperpolarised, and the noise reduced (Fig. 2c). With downward movements it is depolarised, and the noise increased (Fig. 2d). Clockwise (Fig. 2e), or counter-clockwise (Fig. 2f) horizontal movements are ineffective. The movement responses are largest at a pattern speed of 2 periods per s, thus corresponding to the velocity-dependent optomotor behaviour of intact flies<sup>11-13</sup>. It is possible to record this kind of electrical activity from any one of the VS- or HS-cells for more than 1 h, without ever



**Fig. 1** Neurone VSI of the blowfly *Calliphora erythrocephala*, as reconstructed after procion yellow injection, and serial sectioning. The right half of the fly's brain is shown from behind. Neuropil areas are white, tracts and perikaryon layers are shaded. The retinotopic projection of the ipsilateral visual field into the plane of the third visual neuropil (lobula plate) is indicated. VSI belongs to a set of 10 large movement sensitive neurones, which occupy the caudal face of the neuropil. Their dendritic arborisations are in the lobula plate, the somata are in the caudal surface layer, and their axon terminals are close to the oesophageal canal (EC). Field axes: a-p, antero-posterior (horizontal); d-v, dorsoventral (vertical); arrow: site of penetration.



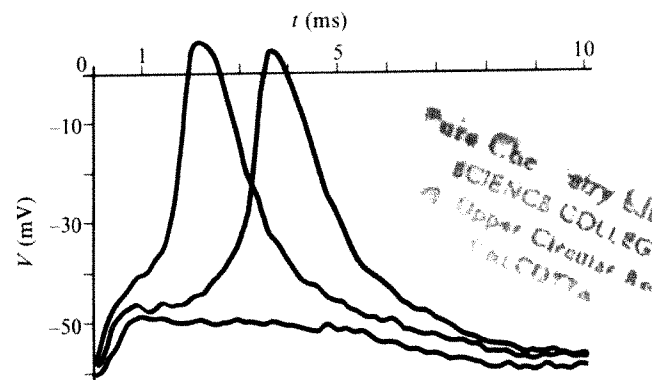
**Fig. 2** Responses of neurone VS1 to visual stimulation. *a*, In darkness the membrane potential is  $V = -41$  mV, and the average noise is 0.6 mV r.m.s. *b*, With constant illumination  $V$  shifts by +2.4 mV, but the noise increases threefold (1.8 mV r.m.s.). *c-h*, Responses to pattern movements. The pattern is illuminated throughout, and starts moving at the broken line. *c*, Upward movement hyperpolarises by  $-8.4$  mV, and reduces the noise; *d*, downward movement depolarises by 11.0 mV, and increases the noise; *e*, clockwise, or *f*, counterclockwise horizontal movements give no significant responses. No spike is present in *a-f*. With steady hyperpolarisation, by injection of  $-2.4$  nA, upward movement (*g*) still elicits a graded hyperpolarising response ( $-7.6$  mV), but the effect of downward movement (*h*) is drastically different from (*d*): as well as an increased average depolarisation (+17 mV) fast spikes of about 30 mV amplitude are generated. These cannot be elicited by steady injection of depolarising current. Stimulus field:  $45^\circ$  lateral,  $60^\circ$  diameter; pattern: grating of 10° period; speed:  $20^\circ \text{ s}^{-1}$ ; brightness:  $50 \text{ cd m}^{-2}$ , contrast: 80%.

observing a full-sized action potential. No spikes are elicited by various other kinds of visual stimuli (such as intense flashes, discrete moving objects) or by depolarising currents up to 10 nA.

VS- and HS-cells, like the neurone VS1 shown here, apparently have the specific properties of 'non-spiking' interneurons<sup>2</sup>, namely: (1) small average membrane potential ( $-30$  mV to  $-50$  mV), (2) conspicuous noise-like potential fluctuations, (3) graded response to stimulation, (4) inability to generate action potentials with imposed depolarisation, and (5) graded transmission across chemical synapses of either polarity. This last property could not be tested here by simultaneously recording from postsynaptic neurones, but estimated length constants in VS and HS axons would allow graded potential changes to spread from the dendritic arborisation to the axon terminals. Any VS or HS cell will, however, generate spikes, if it is steadily hyperpolarised by current injection. The current required varies from less than  $-1$  nA to about  $-5$  nA and is inversely correlated with the membrane potential before current injection. The records in Fig. 2*g, h* are from the same neurone, VS1, as those in Fig. 2*a-f*, with the only difference that  $-2.4$  nA are continuously injected through the recording microelectrode by means of a balanced bridge circuit. The cell responds to pattern movements as before, but Fig. 2*h* shows in addition fast spikes which often ride on top of small depolarising voltage fluctuations. These

spikes have a fast repolarising phase, their amplitude increases with current and may overshoot ground potential. The current-induced hyperpolarisation of the cell membrane does not exceed the equilibrium potential of the inhibitory synaptic ion channels: as Fig. 2*g* shows, there is still a hyperpolarising response. The current effect is reversible, and with steady injection, the generation of spikes may continue for more than 2 h. These findings were repeatedly confirmed for 6 of 10 VS cells, and for 2 of 3 HS cells. Figure 3, for instance, shows spikes from the neurone HS1 which are elicited by excitatory post-synaptic potentials (e.p.s.p.s), but only if the cell is hyperpolarised. The same effects are expected from the remaining 6 neurones in the two classes, and are independent of the electrode-filling solution (5% Procion yellow M4RAN, 5% Procion rubine MX-B, isotonic potassium chloride).

The relationships between injected current, membrane potential, spike rate and spike amplitude, as studied more extensively in a VS neurone, are shown in Fig. 4. With increasing hyperpolarising current the spike rate rises rapidly from  $-1.5$  nA to  $-3.0$  nA, and declines again to zero between  $-3.0$  nA and  $-5.5$  nA, although the stimulation was stationary (constant light). The average membrane potential changes from  $-30$  mV at 0 nA to  $-60$  mV at  $-5.0$  nA. The spike amplitude increases non-linearly with current, and overshoots ground potential if more than  $-3.0$  nA are injected. The significant features of these spikes, namely their large amplitude, positive peak potential, and fast rate of repolarisation are not likely to arise from either chemical or electrical synaptic potentials, charging an otherwise electrically passive membrane.



**Fig. 3** Overshooting action potentials from neurone HS1, during steady hyperpolarisation with  $-1.0$  nA. The spikes are elicited by large e.p.s.p.s, evoked by horizontal pattern movement in the contralateral visual field. In one of the three superimposed traces no spike is generated and the e.p.s.p. is clearly visible. Records were taken at  $5^\circ \text{C}$  in order to spread the membrane processes in time, thereby circumventing the lowpass characteristic of the microelectrode ( $240 \text{ M}\Omega$ ; 5% procion yellow M4RAN).

Rather, they demonstrate the existence of voltage-controlled conductance channels with kinetic properties appropriately tuned to produce regenerative action potentials. Accordingly Fig. 4 indicates that the current range of more than  $-5$  nA corresponds to the resting state in a conventional neurone,  $-2$  nA to  $-5$  nA to an excited state, whereas between 0 and  $-2$  nA the membrane is refractory. In a schematised notion of electrical excitability this means that most sodium channels are inactivated, and a large proportion of potassium channels are open. This state would be unstable unless the resulting outward potassium current is balanced by an inward current of equal strength. Either leakage or a constant and strong excitatory synaptic input could provide the inward current. Switching off the stimulating light reduces the synaptic 'noise' (Fig. 2*a*) without significant increase in membrane potential. The more



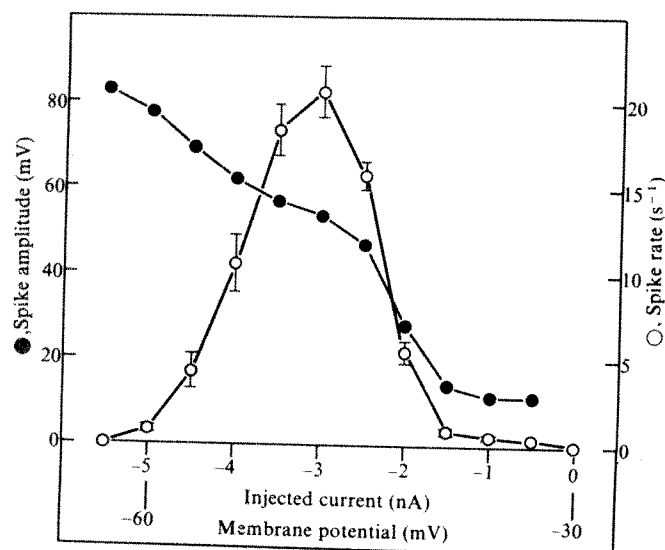


Fig. 4 Spike rate (○) and spike amplitude (●) as functions of injected current in a VS neurone of *Calliphora*. Weak stationary excitation is provided by constant illumination (Fig. 2b). With increasing hyperpolarisation of the cell membrane, the spike rate ( $\pm$  s.e.m.) rises and falls steeply. At  $-5.0$  nA the membrane potential is  $-60$  mV, and the spike amplitude of  $80$  mV overshoots ground potential. The dependence of spike rate on membrane polarisation, the overshoot, and the fast repolarising phase (Fig. 3) characterise regenerative action potentials.

probable cause of the depolarised state is, therefore, a steady inward leakage current.

An interesting consequence with respect to the 'noisy' appearance of the membrane potential in the non-spiking state arises from the rapid breakdown of spike rate and spike amplitude between  $-2.5$  and  $-1.5$  nA in Fig. 4. In this poorly-defined state of the regenerative mechanism small fluctuations of synaptic or leakage currents will be amplified into comparatively large potential fluctuations. Noise measurements in this experiment yielded  $0.78$  mV r.m.s. at  $0$  nA,  $0.75$  mV r.m.s. at  $-5.5$  nA, but  $2.8$  mV r.m.s. at  $-2.0$  nA, where recognisable spikes contribute less than  $0.3$  mV r.m.s. to the overall noise.

These results show that VS and HS neurones have electrically excitable membranes. Their graded mode of operation is due to refractoriness of the regenerative membrane mechanism. Consequently neither natural nor imposed depolarisation will elicit spikes. The conspicuous membrane potential 'noise', which characterises the non-spiking state, is mainly caused by abortive membrane excitation. It is not clear why these cells in particular should be refractory, whereas other cells generate spikes. The advantage of permanent refractoriness is questionable; moreover, it has been shown in *Limulus* that electrical excitability can be differentially damaged in closely adjacent cells<sup>14</sup>. The 'non-spiking' state may therefore not represent the natural condition of information processing in these neurones.

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- Schmitt, F. O., Dev, P. & Smith, B. H. *Science* **193**, 114–120 (1976).
- Pearson, K. G. & Fuortner, C. R. *J. Neurophysiol.* **38**, 33–52 (1975).
- Burrows, M. & Siegler, M. V. *S. Nature* **262**, 222–224 (1976).
- Chappell, R. L. & Dowling, J. E. *J. gen. Physiol.* **60**, 121–147 (1972).
- Laughlin, S. B. *J. comp. Physiol.* **112**, 199–212 (1976).
- Zettler, F. & Järvilehto, M. *J. comp. Physiol.* **85**, 89–104 (1973).
- Dvorak, D. R., Bishop, L. G. & Eckert, H. E. *J. comp. Physiol.* **100**, 5–23 (1975).
- Hausen, K. *Z. Naturforsch.* **31c**, 629–633 (1976); thesis, Univ. Tübingen (1976).
- Pierantoni, R. *Cell. Tiss. Res.* **171**, 101–122 (1976).
- Strausfeld, N. J. *Atlas of an Insect Brain*, Table 7.29 (Springer, Heidelberg, 1976).
- Götz, K. G. *Kybernetik* **2**, 77–92 (1964); **4**, 199–208 (1968).
- Götz, K. G. & Wenking, H. *J. comp. Physiol.* **85**, 235–266 (1973).
- Reichardt, W. & Poggio, T. *Q. Rev. Biophys.* **9**, 311–438 (1976).
- Barlow, R. B. & Kaplan, E. J. *gen. Physiol.* **69**, 203–220 (1977).

## Evolutionary adjustment of developmental time in mixed populations of flour beetles

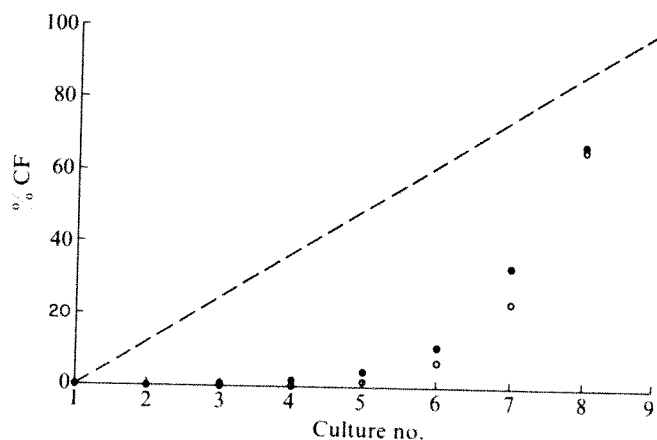
IN dense, synchronised laboratory populations of the flour beetle *Tribolium castaneum* (referred to here as CS), there is a substantial amount of cannibalism of the earliest pupating individuals by large larvae, thus creating the possibility of strong selection pressure against fast development<sup>1–3</sup>. An opportunity to investigate this possibility was provided by the existence of a set of mixed-species cultures that had been maintained for several years. These cultures contain CS and *T. confusum* (CF) in varying proportions. Since developmental time of CF is from 1–2 d longer than CS<sup>4</sup>, the null hypothesis of no differences in developmental time of CS from the various cultures could be tested against the alternative that developmental time of CS would increase as the proportion of slower developing CF in the culture increases. I report here that developmental times do in fact adjust in mixed populations of these flour beetles.

As part of a study of changes in competitive ability and other fitness components in populations of CS exposed to differing proportions of intra- and interspecific 'competition', two replicate series of nine cultures were established in 1968 using beetles from new, genetically heterogeneous stocks of CS and CF. The cultures are maintained at  $29^{\circ}\text{C}$ , 50–70% relative humidity in half-pint milk bottles containing about 30 g of whole wheat flour enriched with 5% brewer's yeast. At the beginning of each transfer, or cycle of selection, the nine cultures receive 200 adults, distributed among the two species as follows: culture number (1), 200 CS/0 CF; (2), 175 CS/25 CF; (3), 150 CS/50 CF; (4), 125 CS/75 CF; (5), 100 CS/100 CF; (6), 75 CS/125 CF; (7), 50 CS/150 CF; (8), 25 CS/175 CF; (9), 0 CS/200 CF. The initial adults are discarded 3 weeks after being placed in the cultures, thus insuring a minimum of one generation in each transfer. About 7 weeks later, the cultures are censused and the initial composition is re-established.

In these conditions CF is clearly inferior to CS; Fig. 1 shows that the percentage of CF recovered is always lower than the percentage used to initiate the cultures. In fact, only cultures 8 and 9 (often 7 and occasionally 6) produce enough CF adults to re-establish the initial number. The CF beetles used to reinitiate the deficient cultures come from cultures 8 or 9.

Developmental time was measured in the summer of 1973 on the progeny of beetles taken from the 28th transfer using standard procedures<sup>4</sup>. For each culture in each of the two series, two replicate vials were set up containing 150 eggs 0–24 h of age in 6 g of flour–yeast medium. Numbers of pupae were recorded by sex at daily intervals starting 18 d after egg laying.

Fig. 1 % CF recovered from cultures 1–9 of the two series, compared to the % used to initiate the cultures (dotted line) for the first three transfers, or cycles of selection. Open and closed circles represent the two replicate series of cultures.



Since developmental time is not normally distributed<sup>4</sup>, analyses were done on vial means, which were based on from 98 to 137 individual observations.

Developmental time data are shown in Fig. 2. Cultures 8 are obviously very different from cultures 1–7. Ignoring cultures 8, a factorial analysis of variance was carried out using the vial means from cultures 1–7. Main effects were series (2), cultures (7) and sexes (2). The only significant main effect was cultures ( $F = 9.88$ ,  $P < 0.001$ ). The culture  $\times$  series interaction was significant at the 0.05 level; this effect is a real one as will be shown below. Although differences between males and females have been found in some studies<sup>4</sup>, they were not evident here. Sexes were pooled for subsequent analyses.

Regression of developmental time on culture number for cultures 1–7 was carried out separately for each series. Linear regression was a significant source of variation for both series, and in one case there were also significant deviations from regression. The test for equality of slopes of the two regression lines showed that the slopes were not different, so a common line is plotted in Fig. 2. There is clear evidence for an increase in developmental time as a function of culture number.

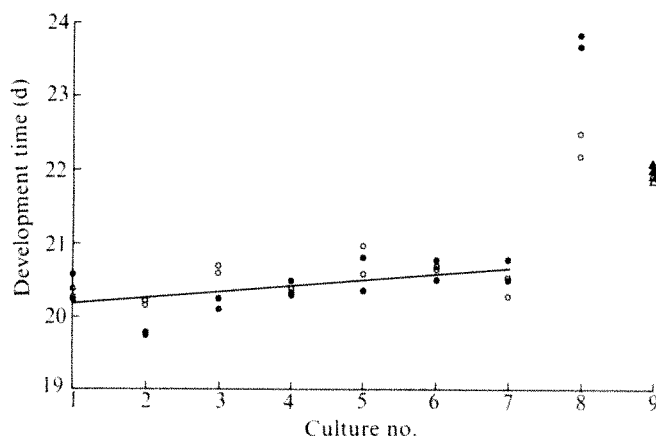


Fig. 2 Developmental time (from egg to pupa) for CS from cultures 1–8 and CF from culture 9. Data points are means of 98–137 individuals. Open and closed circles represent the two replicate series of cultures. The line from cultures 1–7 is a common regression line for the two series. The regression equation is  $y = 20.12 + 0.08x$ .

Turning now to cultures 8; developmental times changed dramatically to the point where they are even slower than the CF controls in cultures 9. It might be argued that this is a result of inbreeding rather than natural selection, since cultures 8 are reinitiated with only 25 adults at each transfer and developmental time is known to be subject to severe inbreeding depression in CS<sup>5</sup>. I argue here, however, that the change is an adaptive one.

First, inbreeding depression also leads to a great increase in variability of developmental time<sup>5</sup>, as does artificial selection for slow development<sup>4</sup>. Coefficients of variation for developmental time in controls (cultures 1) and cultures 8 were 5.84 and 7.78 for series A, and 8.68 and 8.33 for series B; clearly in series B at least the coefficient of variation is not larger in culture 8. This argues against inbreeding depression.

Second, fecundity is known to be also subject to severe inbreeding depression in this species<sup>6</sup>. Fecundity data, obtained in separate egg cannibalism experiments, will be published elsewhere. In one of the two series, the fecundity of females from culture 8 was not lower than that of the other populations, and in the other series there was a linear decrease in fecundity with culture number. These results do not support the hypothesis of inbreeding depression.

Finally, note that cultures 8 are the only ones where CF always replaces itself; 70–90% of the surviving adults at the time of census are CF. In addition, there is a marked similarity

between Figs 1 and 2. Pearson product-moment correlation coefficients were calculated for developmental time versus percentage CF for both the 1st and the 28th transfers (Table 1). The correlations for transfer 1 are both significant, in agreement with the hypothesis. Most remarkable, however, are the extremely high correlations at transfer 28. In the two series, 94 and 99% of the between-culture variation in developmental time can be accounted for by differences in the percentage CF among surviving adults.

Table 1 Coefficients of correlation ( $r$ ) and determination ( $r^2$ ) between mean developmental time in CS and competitive ability

Series		Transfer 1	Transfer 28
A	$r$	0.76*	0.97†
	$r^2$	0.57	0.94
B	$r$	0.89†	0.99†
	$r^2$	0.79	0.99

Competitive ability was measured as % CF among surviving adults.

\* $0.05 > P > 0.01$ .

† $P < 0.01$ .

The hypothesis that developmental time of CS should increase as the proportion of slower developing CF in the cultures increases is supported by my data. The proposed mechanism of selection for slow development is cannibalism of early pupating individuals by large larvae. This mechanism was first suggested by Sokal and Sonleitner<sup>1</sup> as an explanation for increased developmental time in experimental CS populations. Using an artificial mixture of genotypes, I have shown that in dense cultures of CS there was strong selection against early pupation<sup>3</sup>. With increasing culture number in the present experiments there is a linear increase in the proportion of CF adults used to initiate the culture, but a distinctly non-linear increase in the proportion of CF among the survivors (Fig. 1). Developmental times of CS from these cultures form a very similar non-linear curve (Fig. 2). The high correlations between developmental time and percentage CF in the cultures at the time they were first established might conceivably be spurious ones involving some other, unknown, selective agent. But the extremely high correlations at transfer 28 are clearly suggestive of an adaptive response.

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1. Sokal, R. R. & Sonleitner, F. J. *Ecol. Monogr.* **38**, 345–379 (1968).
2. Wool, D. *Res. Pop. Ecol.* **11**, 40–44 (1969).
3. Dawson, P. S. *Genetics* **80**, 773–783 (1975).
4. Dawson, P. S. *Genetics* **51**, 873–885 (1965).
5. Dawson, P. S. *Evolution* **22**, 217–227 (1968).

## Bismuth staining of Golgi complex is a characteristic arthropod feature lacking in *Peripatus*

THE traditional view for the origin of arthropods is that an exoskeleton has evolved once and the three major modern groups, Crustacea (for example, crabs), Chelicerata (spiders) and Uniramia (insects) had a common ancestor in the pre-cambrian. This ancestor was itself related to primitive Onychophora (present day representative, *Peripatus*) and ultimately to predecessors of the annelids<sup>1–4</sup>. But, arthropods may have had two, three or several separate evolutionary origins if the external features thought to unite the groups have evolved in response to functionally similar requirements<sup>3,5</sup>. Studies on



trilobite skeleton, for example, suggest two independent ancestral groups whose members were not themselves arthropods. These gave rise on the one hand to the Uniramia and on the other to the chelicerates which were linked to Crustacea through ancestral trilobites<sup>6</sup>. The discovery of a cell component characteristic of all cell types in insects<sup>7</sup> prompted a systematic study of its distribution, to see if it could be of help in determining arthropod evolutionary relationships. We report here that a characteristic feature of arthropods, bismuth staining of the Golgi complex beads, is absent in *Peripatus*, thus supporting the established view of arthropod evolution.

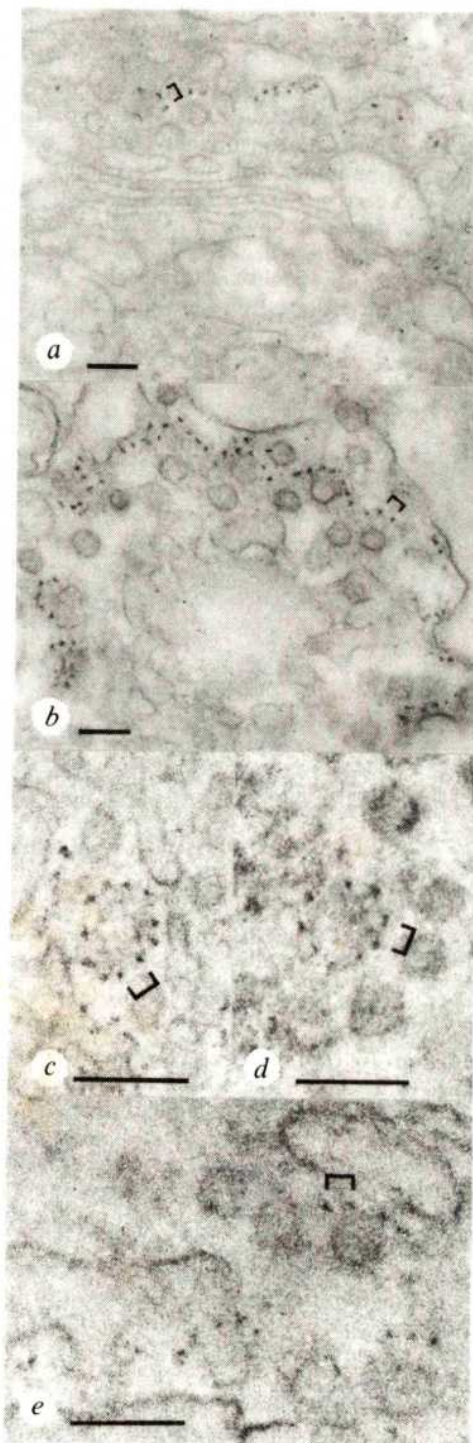
The smooth surface of the rough endoplasmic reticulum that makes the forming face of the Golgi complex in all cell types in *Calpodes* (Lepidoptera, Hesperidae), has particles arranged like beads on a circular string with about 10 beads per ring and a centre-to-centre spacing of 27 nm. Each particle has a core 11 nm in diameter that stains specifically with bismuth. The rough endoplasmic reticulum membrane distends through the bead rings to form the transition vesicles of the Golgi complex. Particles with the distribution described for *Calpodes* may be present in most Golgi complexes but unless they stain with bismuth they are difficult or impossible to resolve. In some vertebrate tissues, for example, particles with the appropriate distribution do not react with bismuth and can only just be resolved after uranyl staining<sup>8</sup>. These results suggested that the beads or functionally comparable structures may be a general feature of Golgi complexes in organisms and that the bismuth staining might have a meaningful but restricted phylogenetic distribution. We therefore surveyed a number of arthropods and their near allies to determine the distribution of the bismuth staining of beads.

Tissues from organisms in the phyla listed in Table 1 were fixed in glutaraldehyde, stained only in bismuth solution before embedding<sup>9</sup> and sections viewed with a Philips 300 electron microscope at a magnification of 250,000 and kept within 400 nm of focus. A piece of *Calpodes* tissue was carried through with each test specimen to make sure that failure to stain was not caused by an undetected failure of the technique. The success of the bismuth staining could also be judged by the reaction with interchromatin and perichromatin granules present in most nuclei. In practice there was rarely any doubt whether stained beads were present on suitably prepared Golgi complexes. They were usually found within minutes of beginning an observation or not at all, even after hours or days of searching. It may be that modification of the staining procedure could stain beads unrecognised hitherto, but the variations in staining recorded here reflect a consistent difference not attributable to variations in procedure.

**Table 1** Groups in which bismuth-stained Golgi complex beads are present (+) and absent (—)

Cnidaria		( <i>Condylactis</i> , <i>Hydra</i> )	—
Platyhelminthes		( <i>Bipalium</i> , <i>Pseudoceros</i> , <i>Planaria</i> )	—
Nematoda		( <i>Phoconema</i> )	—
Mollusca		( <i>Placopecten</i> , <i>Anodonta</i> )	—
Annelida		( <i>Spirorbis</i> , <i>Sabella</i> , <i>Tubifex</i> )	—
Onychophora		( <i>Epiperipatus</i> )	—
Tardigrada		( <i>Hypsibius</i> )	+
Arthropoda	Pycnogonida	Pantopoda (Undetermined)	+
	Chelicerata	Xiphosura ( <i>Limulus</i> )	+
		Acarina (Parasitidae)	+
		Pseudoscorpionidea (Undetermined)	+
	Crustacea	Isopoda (Undetermined)	+
		Decapoda ( <i>Orconectes</i> )	+
	Diplopoda	Polydesmoidea (Undetermined)	+
	Insecta	Orthoptera ( <i>Locusta</i> )	+
		Hymenoptera ( <i>Apis</i> )	+
		Coleoptera ( <i>Tenebrio</i> )	+
		Lepidoptera ( <i>Calpodes</i> )	+

Figure 1 shows some of the results. The characteristic size and spacing described for bismuth-stained GC beads in *Calpodes* is a constant feature throughout the arthropods. Table 1 shows the phylogenetic distribution. The beads are present in all three major arthropod groups and also in pycnogonids and tardigrades. They are absent from Onychophora (*Epiperipatus*),



**Fig. 1** Bismuth staining of the Golgi complex beads in the main arthropod lines of evolution. *a*, Uniramia, Insecta, *Calpodes*, Lepidoptera, Hesperidae, larval epidermis. *b*, Crustacea, Decapoda, *Orconectes* x-organ. *c*, Chelicerata, Arachnida, Acarina, epidermis. *d*, Pycnogonida, Pantopoda, epidermis. *e*, Chelicerata, Merostomata, Xiphosura, *Limulus*, epidermis. The tissues were stained only by bismuth which reacts with particles about 10 nm in diameter arranged in rings upon the smooth face of the rough endoplasmic reticulum where transition vesicles of the Golgi complex arise. The square bracket marks the characteristic 27 nm spacing. Stained beads are absent from all tissues in *Epiperipatus*. Scale bar, 100 nm.



annelids, molluscs and flatworms. The inclusion of the tardigrades with arthropods agrees with work showing chemical similarities between the cuticles<sup>10</sup>. The distribution of beads thus favours a common origin for present-day arthropods and makes it unlikely that the Uniramia arose separately from a *Peripatus*-like ancestor.

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1. Clarke, K. U. *The Biology of the Arthropoda* (eds Barrington, E. J. W. & Willis, A. J.) 172 (Edward Arnold, London, 1973).
2. Imms, A. D. *Outlines of Entomology* 167–170 (Methuen, London, 1942).
3. Manton, S. M. *Zool. J. Linn. Soc.* 51, 380 (1972).
4. Meglitsch, P. A. *Invertebrate Zoology* 668 (Oxford University Press, New York, 1967).
5. Tiesie, O. W. & Manton, S. M. *Biol. Rev.* 33, 255–337 (1958).
6. Cisne, J. L. *Science* 186, 13–18 (1974).
7. Locke, M. & Huie, P. J. *Cell Biol.* 70, 384–394 (1976).
8. Locke, M. & Huie, P. *Tissue Cell* 8, 739–743 (1976).
9. Locke, M. & Huie, P. *Tissue Cell* 9, 347–371 (1977).
10. Jeuniaux, C., *Cah. Biol. mar.* 16, 597–612 (1975).

## Induction of fibrin thrombi by monocytes

THE discovery that blood clots in the absence of its cellular components has suggested that coagulation is independent of blood cells<sup>1</sup>. This view has been modified by the demonstration of procoagulant factors in the platelets<sup>2</sup> and anticoagulant heparin in basophils<sup>3</sup>. Procoagulant activity has also been found in extracts of leukocytes<sup>4,5</sup>, showing that coagulation is a cell-regulated phenomenon. We report here the direct observation of focal clotting induced by specifically activated monocytes.

Orientated fibrin micro-clots can be produced by incubation of bacterial endotoxin, the white cell buffy coat and heparinised plasma from sensitised patients<sup>6</sup>. The fibrin radiates in a stellate pattern from a centre of indeterminate nature (Fig. 1). We have analysed this central body.

When we used buffy coats rendered largely devoid of platelets by repeated centrifugal separation, the nucleus of the micro-thrombus appeared as a cell rather than an amorphous mass. Attempts to ascertain the responsible cell by using Ficoll, dextran and albumin separation techniques were unsuccessful because thrombi did not form. But direct centrifugal separation of untreated buffy coat specimens in plastic tubes<sup>7</sup> revealed the upper portion of the buffy coat containing lymphocytes and monocytes to be associated with the induction of the greatest number of thrombi. The lower fraction containing the heavier polymorphonuclear leukocytes showed minimal activity. Rectangular capillary tube thrombi counts<sup>8</sup> and differential counts of smears of comparable buffy coat fractions demonstrated a correlation between the number of thrombi and the number of monocytes or lymphocytes, but not the number of neutrophils, eosinophils or basophils.

The monocyte shows strong surface adherence in contrast to the lymphocyte, and we found that the cells responsible for the thrombi rapidly attached to glass. Buffy coat preparations left in contact with the glass capillary tube or on glass cover slips could be flushed or washed away after 30–60 min at 37 °C, leaving adherent cells which became centres for thrombus formation in the endotoxin-plasma mixture then applied. Furthermore, in preparations made on cover slip slides whereby the slip and slide were forced apart, the central cell had a remarkable set of pseudopods, as evidence of its strong attachment to both surfaces (Fig. 2). Blood left in contact with glass wool for 1 h and then removed failed to produce thrombi.

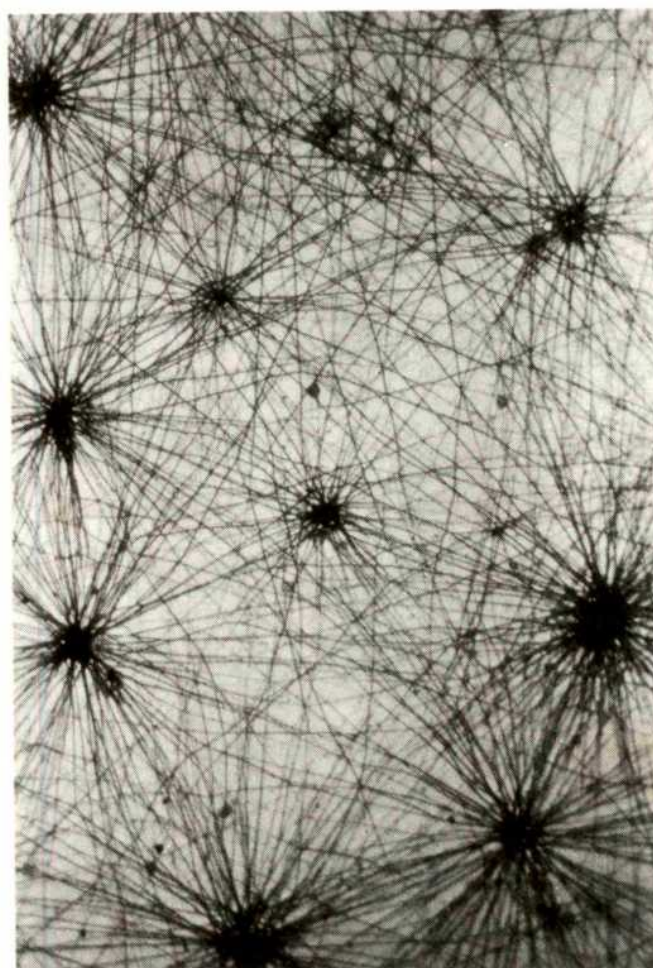


Fig. 1 Multiple stellate foci of fibrin induced by addition of lipopolysaccharide W ( $10^{-4}$ ) to heparinised blood from patient with vasculitis. The cover slip preparation was incubated 18 h at 37 °C. Central bodies appear to be of indeterminate nature. (Wright-Giemsa.)

Conversely lymphocyte specimens (99% pure) obtained by passing blood through cotton<sup>8</sup> produced no thrombi when exposed to endotoxin.

The cells responsible for the thrombi also proved to be phagocytic. The addition of latex particles<sup>9</sup> (0.011  $\mu$ m, 50  $\lambda$  of  $10^{-4}$  in 1 ml blood) to the regular system led to their endocytosis by the central cell of many of the thrombi (Fig. 3).

Phase and phase interference microscopy with unsealed cover slip preparations showed that the centre of the thrombus was a large cell, generally without refractile granules, and with a reniform, pyknotic or vacuolated nucleus. The cell appearing to be a monocyte had fine filopodia at first. The earliest identifiable pericellular fibrin crystals appeared after 3.5 h. These were short and increased in number and length as time passed. The cell had grey cytoplasm and the nucleus became pyknotic, at time showing karyorrhexis and perinuclear vacuole formation. It sometimes ingested granules. The cell enlarged, became degenerate<sup>10</sup>, with cytoplasm of granular appearance, within 24 h (Fig. 3). At the end of this time, cell identification proved difficult in most cases because of the advanced degenerative changes. The spreading of the central cell could be accentuated markedly by placing a 30-g weight on the cover slip during incubation. Wright-Giemsa (5% acetic acid methanol fixation) or haematoxylin-eosin (Carnoy's fixative) stained the nucleus clearly and showed an eosinophilic cloud at the centre of the thrombus. The myeloperoxidase<sup>11</sup> stain (10% formalin alcohol) showed fine granules consistent with those seen in the monocyte, and neutral red staining showed supravital uptake by some of the central cells. The best stain proved to be the Papanicolaou<sup>12</sup> (95% ethyl alcohol fixation)



because it stained the cytoplasm as well as the nucleus. In the early phase the cytoplasm was green, but after about 6 h it was a distinctive pink orange and later yellow. This seemed to herald the death of the cell, and with this stain each thrombus could be readily spotted by the pinkish centre.

Immunofluorescent staining with fluorescein-isothiocyanate-conjugated anti-human immunoglobulins (Cappel) of cold-acetone-fixed cover slip specimens revealed the central cell stained with  $C_3$  but not with IgG A M D or  $C_2$  or  $C_2$ . This is consistent with the fact that the monocyte has a receptor for  $C_3$  (ref. 13).

The monocyte seems to have a clear cellular role in coagulation. Its ability to induce focal fibrin formation may be related to the observation that human monocytes exposed for 4 h at 37 °C to *Escherichia coli* lipopolysaccharide have a demonstrable coagulant effect in a one-stage coagulation assay<sup>14</sup>. Other non-specific effects of endotoxin include the release of pyrogen, as well as T-cell and colony stimulation<sup>15</sup>. This is in contrast to the specific phenomenon we have observed in patients with vasculitis and various immunological diseases. The fibrin deposition which we have observed around the monocyte may reflect complement activation through the alternative pathway.

As a result of these studies, the monocyte can be viewed as a clot cell, in Yin-Yang contrast to the heparin-containing basophil, an anticlot cell. Study of the monocyte in cell-mediated coagulation promises new information about thrombosis, gram-negative infections and various immunological diseases which involve fibrin deposition. So far the only clinical analogue of our laboratory micro-thrombi may be the stellate

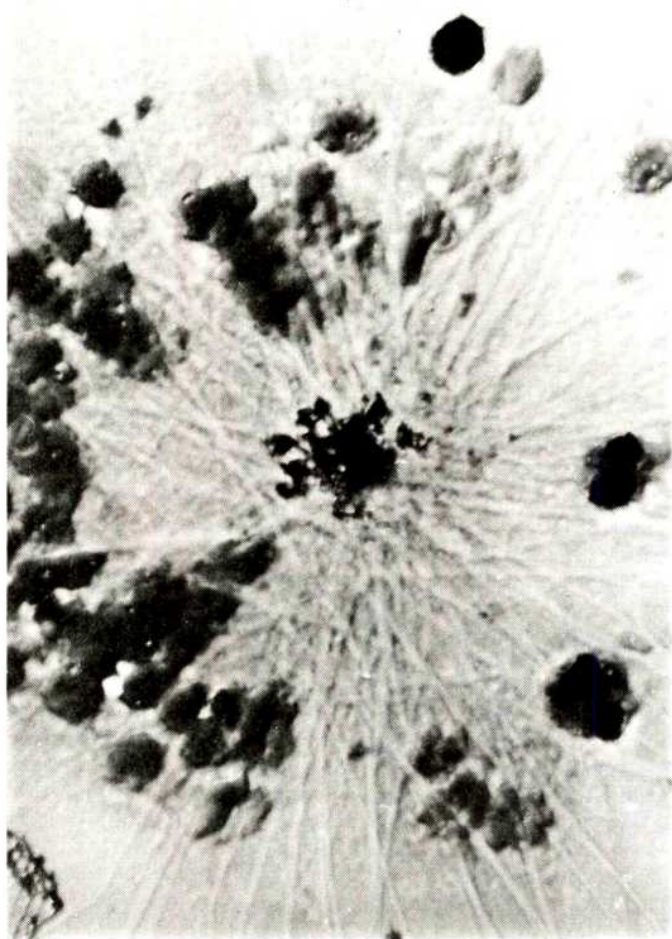


Fig. 2 Single stellate micro-thrombus forming in presence of buffy coat from which platelets have been removed. The unstained fibrin crystals radiate from monocyte, a central dark staining cell with pseudopods. Note lymphocyte at top two polymorphonuclear cells to right and peripheral red cell clusters. (Wright-Giemsa, phase.)

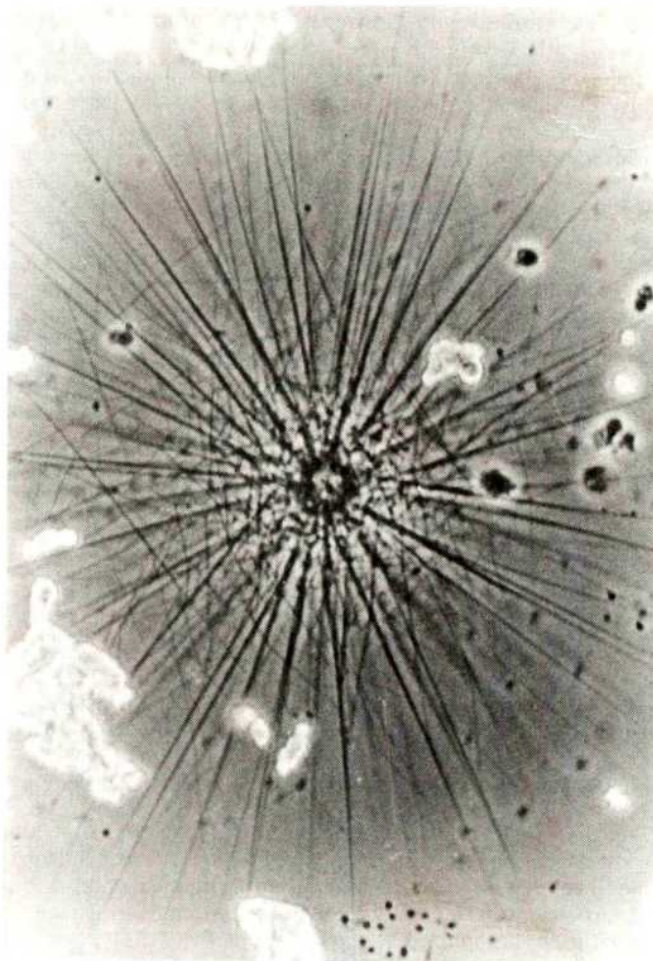


Fig. 3 Single stellate micro-thrombus forming in presence of latex particles. Refractile granules in central cell demonstrate it to be phagocytic. (Unstained, phase.)

fibrin formation observed in the bone marrow of patients with myelofibrosis<sup>16</sup>. The nature of this is not known but the bone marrow monocyte may play a central role.

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1. Tullis, J. L. *Clot* (Thomas, Springfield, Illinois, 1976).
2. Walsh, P. N. *Blood* 43, 597 (1974).
3. Parawaresch, M. R. *The Human Blood Basophil* (Springer, Berlin, 1976).
4. Niemetz, J., Muhlfelder, T., Chierago, M. E. & Troy, B. *Ann. N.Y. Acad. Sci.* 283, 208 (1977).
5. Rothberger, H., Zimmerman, T. S., Spiegelberg, H. L. & Vaughan, J. H. *J. clin. Invest.* 59, 549 (1977).
6. Juhlin, L. & Shelley, W. B. *Lancet* i, 21 (1977).
7. Shelley, W. B. *Am. J. clin. Path.* 39, 433 (1963).
8. Ling, N. R. & Kay, J. E. *Lymphocyte Stimulation* 2nd edn. (North Holland, Amsterdam, 1975).
9. Rothbart, P. H., Hendriks-Sturkenboom, I. & Ploem, J. S. *Blood* 48, 139 (1976).
10. Bessis, M. *Living Blood Cells and Their Ultrastructure* (Springer, New York, 1973).
11. Kaplow, L. S. *Blood* 26, 215 (1965).
12. Keebler, C. M. & Reagan, J. W. *A Manual of Cytotechnology* 4th edn (American Society of Clinical Pathologists, Chicago, 1975).
13. Seligmann, M. *Br. J. Haemat.* 31, Suppl. 1, 1 (1975).
14. Rivers, R. P. A., Hathaway, W. E. & Weston, W. L. *Br. J. Haemat.* 30, 311 (1975).
15. Territo, M. C. & Cline, M. J. *J. Immun.* 118, 187 (1977).
16. Nezelof, C., Soulier, J. P., Griscelli, C. & Royer, P. *Nouvelle Rev. Franc. d'Hematologie* 14, 641 (1974).

## Morphological abnormalities in spermatozoa of man and great apes

HUMAN semen has long been known to differ from that of any other mammal in the high proportion of abnormal spermatozoa that it contains<sup>1</sup>. But there has been almost no opportunity to compare human spermatozoa with those of man's closest living relatives, the chimpanzee (*Pan troglodytes*), the pygmy chimpanzee (*Pan paniscus*), the gorilla (*Gorilla gorilla*) and the orangutan (*Pongo pygmaeus*); these are all now endangered species, and the few animals in zoos are not available for experimentation. Recently, workers at the Yerkes Regional Primate Research Center, where all four species are maintained, have perfected a technique for obtaining semen from these rare animals by electroejaculation<sup>2</sup>. Preliminary studies have suggested that marked morphological similarities exist only between the spermatozoa of the gorilla and man<sup>3</sup>. We examined these similarities and differences in more detail, in the hope that spermatozoal morphology might provide some taxonomic clues about our affinities to the great apes.

Measurements of total dry mass were made with an integrated interferometer (Vickers M-86) for a random selection of 50–100 spermatozoa from each individual in each of the five species. In man and gorilla the dry mass distribution was bimodal with two distinct non overlapping peaks, whereas in the chimpanzees and the orangutan it was unimodal (Fig. 2). When arbitrary units of dry mass were used, the mean value of the major peak in man was 730 compared with 960 in the gorilla. In both species the minor peak represented a dry mass approximately twice that of the major peak. These results are similar to those reported for Feulgen-stained human spermatozoa<sup>4</sup>, and suggest that the two groups represent haploid and diploid spermatozoa. The percentage of diploid spermatozoa in man and gorilla was estimated using a larger sample; 21 diploids were found in 2,000 human spermatozoa (1.05%) and 37 in 2,408 gorilla spermatozoa (1.53%). This difference was not statistically significant ( $P > 0.10$ ). Human spermatozoa had the lowest mean haploid dry mass, and gorilla spermatozoa the highest (Table 2). By extracting DNA with trichloroacetic acid and remeasuring dry mass it could be shown that total dry mass was proportional to DNA content in *Homo sapiens*, *Pan troglodytes*, *Gorilla gorilla* and *Pongo pygmaeus* (Fig. 3). Statistical analysis (Table 2) revealed no signi-

**Table 1** Morphology of spermatozoa of man and the great apes

		<i>Homo sapiens</i> (4 individuals)	<i>Pan troglodytes</i> (3 individuals)	<i>Pan paniscus</i> (1 individual)	<i>Gorilla gorilla</i> (2 individual)	<i>Pongo pygmaeus</i> (2 individuals)
Normal	Modal	73.0	95.5	98.0	71.0	98.5
Abnormal	Large head	2.0	0.3	—	2.3	—
	Small head	0.5	—	0.5	0.8	—
	Tapered head	0.3	0.2	—	1.0	—
	Dense-staining	0.6	0.2	0.5	4.5	—
	Vacuolated head	2.4	3.0	1.0	1.3	0.3
	Irregularly shaped	18.4	0.2	—	16.0	0.8
	Multiple heads	0.5	—	—	0.5	—
	Abnormal midpiece	—	0.3	—	0.5	—
	Cytoplasmic droplets	—	—	—	0.3	—
	Immature cells	2.3	0.3	—	2.0	0.5
	Total	100.0	100.0	100.0	100.2	100.1

Semen samples were obtained from normal healthy individuals of proven fertility except for one *Pongo pygmaeus*. In man and in *Pan troglodytes* samples were obtained by masturbation. In *Pan paniscus*, *Pongo pygmaeus* and *Gorilla gorilla* samples were obtained by electroejaculation as described by Warner *et al.*<sup>2</sup>. Semen samples were washed in 0.9% saline and fixed in methanol: acetic acid, 3:1 as described by Sumner<sup>4</sup>. Some of these slides were stained with Papanicolaou's stain and observed under the light microscope: 200 cells were scored per individual and all results of Table 1 are expressed as percentages.

Table 1 shows that there is a similar proportion of 'normal' (modal) and abnormal forms in the ejaculate of men and gorillas; morphologically, the 'normal' forms of the two species are indistinguishable (Fig. 1). Chimpanzee spermatozoa are very different in appearance to human or gorilla spermatozoa, although very uniform in shape; they seem to be identical in all aspects to the spermatozoa of the pygmy chimpanzee. The spermatozoa of the orangutan are morphologically distinct from all the other species, although once again extremely uniform in shape.

Significant differences in dry mass between individuals within a species, but marked differences between species. Although human spermatozoa are morphologically most similar to those of the gorilla, in terms of DNA content they resemble those of the chimpanzees. Gorilla spermatozoa showed the greatest intra-individual variance in haploid DNA content.

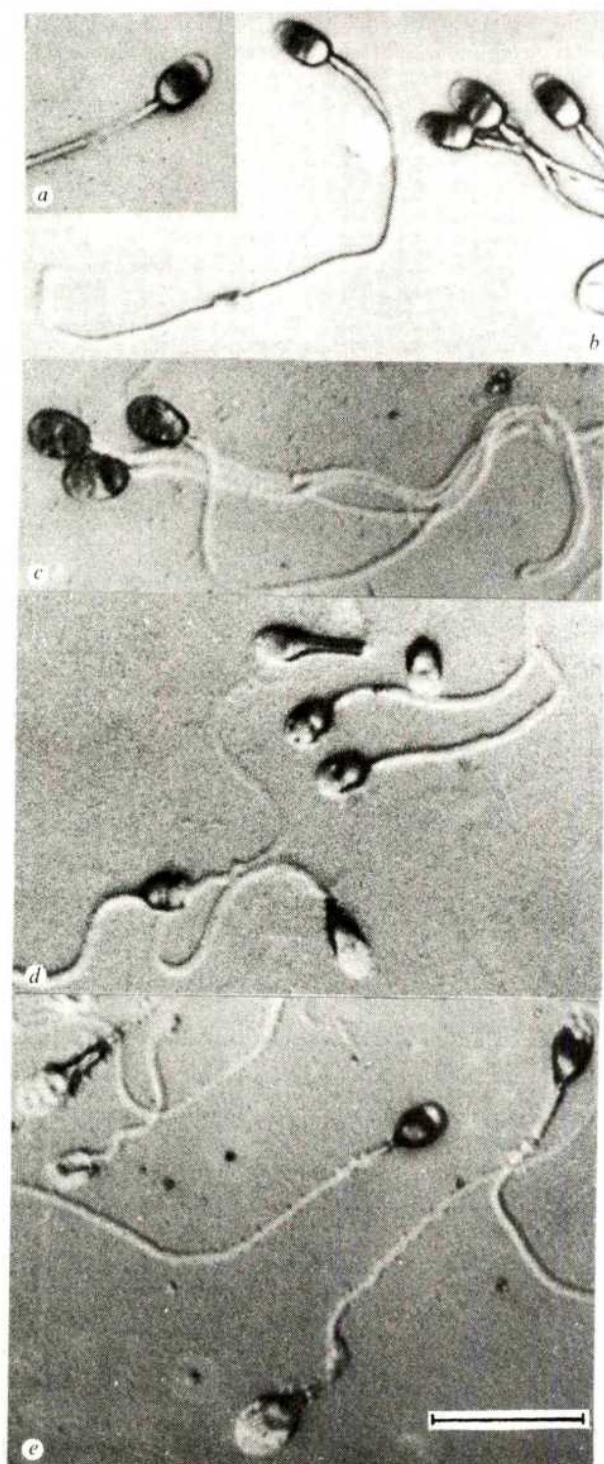
Diploid DNA estimates in the somatic cells of the great apes and man have revealed *Homo sapiens*, < *Gorilla gorilla*, < *Pan troglodytes*, < *Pongo pygmaeus*<sup>5</sup> which does not correspond with our haploid estimates of *Homo sapiens* < *Pan troglodytes*,

**Table 2** Comparisons of total dry mass of spermatozoa of the great apes and man

Species	Means	Difference	Approximate 95% confidence limits			
			Assumption A		Assumption B	
			Lower	Upper	Lower	Upper
Chimpanzee-human	810.0–742.3	+67.7	+21.8	+113.6	+41.8	+93.6
Orangutan-human	871.6–742.3	+129.3	+73.0	+185.6	+97.6	+161.0
Gorilla-human	965.2–742.3	+222.9	+166.4	+279.5	+190.7	+255.2
Orangutan-chimpanzee	871.6–810.0	+61.6	+5.3	+117.9	+29.9	+93.3
Gorilla-chimpanzee	965.2–810.0	+155.2	+98.7	+211.8	+123.0	+187.5
Gorilla-orangutan	965.2–871.6	+93.6	+28.5	+158.9	+56.6	+130.7

The component of variance between individuals within a species is taken to be either 2,000 a.u.<sup>2</sup> (assumption A) or 1,000 a.u.<sup>2</sup> (assumption B). Anovar of haploid dry-mass revealed (1) no significant differences between preparations from the same individual, (2) that the intra-individual variance components for man, chimpanzee and orangutan did not differ significantly (pooled estimate = 4,949.3 with 485 d.f.), but were significantly less than for gorilla (11,606.7 with 197 d.f.). Confidence intervals were constructed using these estimates and taking values of the inter-individual intra-species component to be either 2,000 a.u.<sup>2</sup> (assumption A) or 1,000 a.u.<sup>2</sup> (assumption B). The assumptions correspond to situation in which one individual in 100 can be expected to differ from its species mean by the equivalent of approximately two (A) or one (B) medium-sized chromosomes respectively. The implied degree of aneuploidy suggests that these are overestimates, as are the widths of the corresponding confidence intervals.



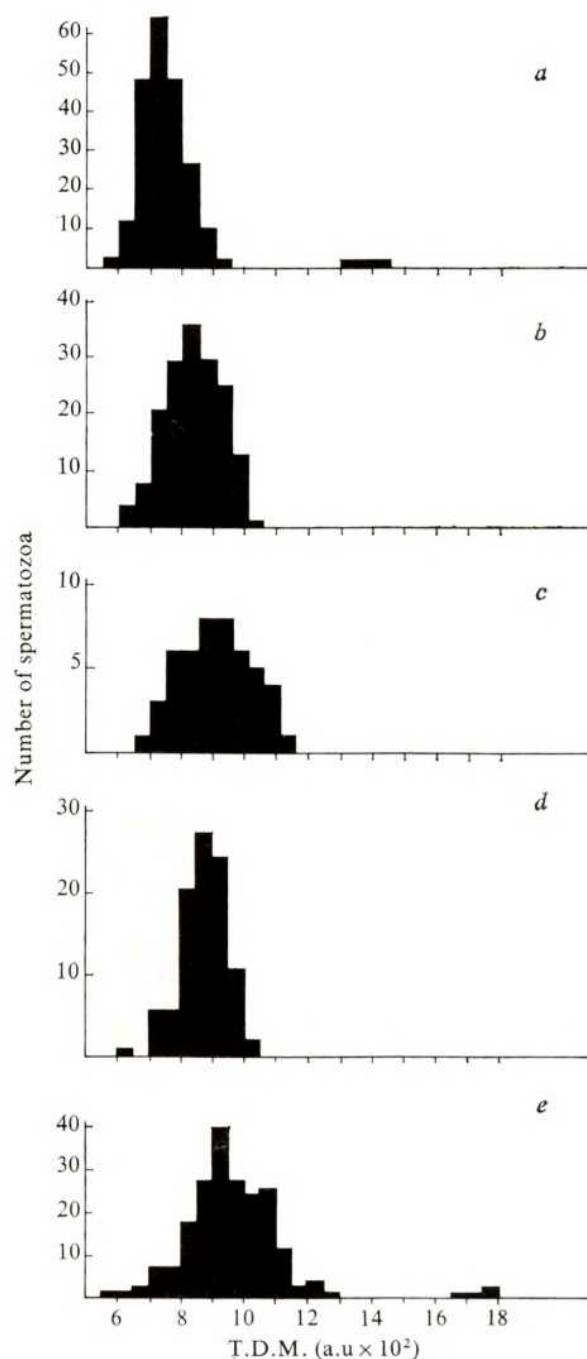


**Fig. 1** The spermatozoa of *Pan paniscus* (a); *Pan troglodytes* (b); *Pongo pygmaeus* (c); *Homo sapiens* (d); and *Gorilla gorilla* (e) photographed under Nomarski interference. Scale bar, 10  $\mu$ m.

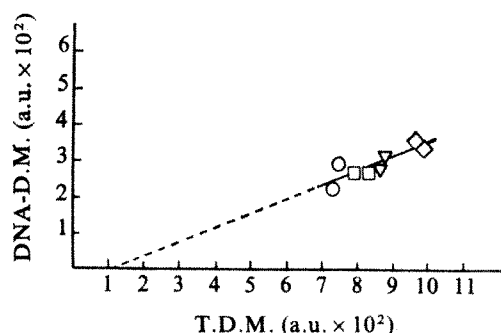
< *Pongo pygmaeus*, < *Gorilla gorilla*. Man has a diploid chromosome number of 46 compared with 48 in all great apes, and less constitutive heterochromatin and brilliant fluorescent chromatin than the chimpanzee and the gorilla<sup>6</sup>. The overall amount of satellite DNA sequences in man seems to be lower than in the great apes<sup>7</sup>, and presumably all these factors contribute to the low DNA content of human cells.

It has been suggested<sup>8</sup> that the pleiomorphism of human spermatozoa may be a consequence of testicular damage caused by clothing-induced hyperthermia. The fact that the spermatozoa of gorillas living in outdoor cages are just as pleomorphic, and

show an even greater variability in haploid DNA content, tends to refute this argument. Furthermore, the great variability in morphology of human spermatozoa, in contrast to the lack of variability in haploid DNA content, means that morphologically abnormal human spermatozoa are not necessarily genetically defective. The percentage of morphologically abnormal forms is increased in infertile men, however, sometimes reaching very high proportions in cases of abnormal chromosome constitution<sup>9</sup>. It



**Fig. 2** The estimations of total dry mass (TDM) were obtained with a Vickers M-86 integrated microinterferometer. This instrument measures the optical path difference (o.p.d. = refractive index  $\times$  thickness) in arb. units (a.u.). The area of the object (the sperm head only) was selected using an electronic masking system. Measurements were obtained with a X 75 n.a. 1.1 water immersion objective and preparations were measured while immersed in distilled water. All semen samples were treated as specified in the legend of Table 1, except that measurements were done on unstained preparations. 50 cells were measured per individual using two slides (25 measurements in each), except in the gorillas (100 cells; 50 measurements per slide). a, *Homo sapiens*; b, *Pan troglodytes*; c, *Pan paniscus*; d, *Pongo pygmaeus*; e, *Gorilla gorilla*.



**Fig. 3** To test whether TDM is proportional to DNA content, 10 spermatozoa from each of two individuals from each species (except *Pan paniscus*) were measured for TDM. After treatment with 5% trichloroacetic acid at 90 °C to remove DNA, the same spermatozoa were relocated and measured for dry mass after extraction (DMAE). Slides were then stained with Feulgen and the same spermatozoa measured for residual DNA with the M-86 instrument in the microdensitometric mode. Only if no DNA was detected were the DMAE values considered valid. The minimum extraction time needed to remove all DNA was 15 min in man and 30 min in the great apes. The difference between TDM and DMAE represents the dry mass of extracted DNA (DNA-DM). The figure shows mean values of DNA-DM and TDM for each individual, and the estimated least-squares regression line. The latter passes close to the origin, suggesting that proportional changes in TDM are identical to those for DNA-DM. (The line should not of course be interpreted as a valid extrapolation beyond the range of the plotted points.) ○, *Homo sapiens*; □, *Pan troglodytes*; ▽, *Pongo pygmaeus*; ◇, *Gorilla gorilla*.

has even been suggested that fertilisation of a defective egg by diploid spermatozoa will give rise to a hydatidiform mole<sup>10</sup>, and that 40% of triploid embryos are a result of fertilisation of a normal egg by a diploid spermatozoon<sup>11</sup>.

Spermatozoal morphology has been shown to be a useful taxonomic guide in assessing relationships between other species<sup>12</sup>. There is already evidence to suggest that man and gorilla are more closely related than any of the other great apes, as judged by their chromosomal karyotypes<sup>13,14</sup>. Our data on the morphology of their spermatozoa lend further support to this conclusion.

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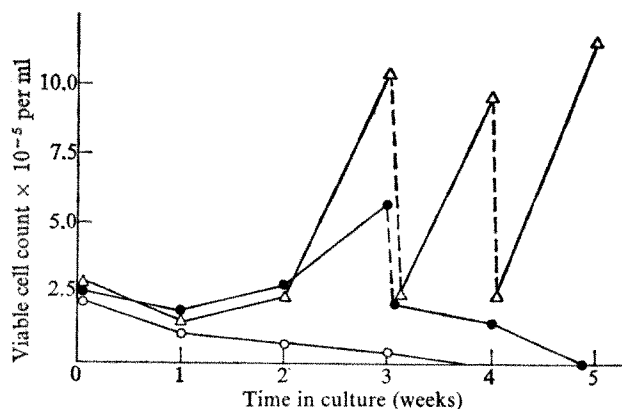
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- Bedford, M. *Contr. Primat.* 3, 97-139 (Karger, Basel 1974).
- Warner, H., Martin, D. E. & Keeling, M. E. *Am. biomed. Engng* 3, 419-432 (1974).
- Martin, D. E., Gould, K. G. & Warner, H. *J. hum. Evol.* 4, 287-292 (1975).
- Sumner, A. *Nature* 231, 49 (1971).
- Manfredi Romanini, M. G. *J. hum. Evol.* 1, 23-40 (1972).
- Conf. Suppl. Standardization in Human Cytogenetics. Birth Defects Original Article Series 1, 9* (The National Foundation, New York, 1975).
- Gosden, J., Mitchell, A., Seuanes, H. & Gosden, C. *Chromosoma* 63, 253-271 (1977).
- Bedford, M., Bent, M. J. & Calvin, H. *J. reprod. Fert.* 33, 19-29 (1973).
- Chandley, A. *et al. Ann. hum. Genet.* 39, 231-254 (1975).
- Kajii, T. & Ohama, K. *Nature* 268, 633-634 (1977).
- Beatty, R. A. *Physiology and Genetics of Reproduction Part A* (ed. Coutinho, A. M. & Fuchs, F.) (Plenum, New York, 1974).
- Baccetti, B. & Afzelius, B. A. *Monographs in Developmental Biology* (Karger, Basel, 1976).
- Seuanes, H., Robinson, J., Martin, D. E. & Short, R. V. *Cytogenet. Cell. Genet.* 17, 317-326 (1976).
- Seuanes, H. *thesis, Univ. Edinburgh* (1977).

## Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture

ATTEMPTS to develop long-term suspension cultures of human myeloid leukaemic cells have met with limited success. Lymphoblastoid lines carrying the Epstein-Barr virus genome occasionally arise during such attempts but these lymphoid cells originate from contaminating B lymphocytes and not from the leukaemic myeloid cells<sup>1</sup>. A line established from the pleural fluid of a patient with chronic myeloid leukaemia in blast crisis<sup>2</sup> (designated K-562) has no B-cell or T-cell markers<sup>3-4</sup> and does not seem to be of lymphoid origin<sup>4</sup>. Its lack of morphological and histochemical differentiation<sup>2-4</sup>, however, makes it difficult to determine whether these cells are derived from myeloblasts or more primitive stem cells<sup>4</sup>. Another less documented cell line (8261) derived from the peripheral blood of a patient with acute myelogenous leukaemia showed apparent morphological and functional differentiation in agar in the presence of a feeder layer of peripheral blood leukocytes but did not differentiate in suspension culture<sup>5</sup>. Our laboratory previously reported that cultures of differentiating myeloid leukaemic cells can be maintained for several months in suspension culture but only when enriched with conditioned media (CM) from certain monolayer fibroblastic cultures of first trimester whole human embryos (ref. 6 and Ruscetti *et al.* in preparation). We describe here for the first time the derivation from myeloid leukaemic cells of a leukocyte culture that by morphological and histochemical criteria clearly and persistently differentiates along the myeloid series without an exogenous source of conditioned medium.

Peripheral blood leukocytes (designated HL-60) from an adult female with acute promyelocytic leukaemia (clinical details of the case will be published elsewhere) were seeded in T30 plastic flasks (Falcon) at  $1.25 \times 10^6$  cells per flask in 5 ml of RPMI-1640 (GIBCO) medium supplemented with 15% heat-inactivated foetal calf serum (Flow Labs) and gentamicin  $50 \mu\text{g ml}^{-1}$ . The flasks were incubated at 37 °C in a humidified 5%  $\text{CO}_2$  atmosphere. A number of the



**Fig. 1** Initial growth of HL-60 leukaemic leukocytes in suspension culture. After seeding the fresh peripheral blood leukocytes at  $2.5 \times 10^6$  cells per ml, weekly haemocytometer counts were done on aliquots of cell suspensions grown in the presence or absence of conditioned media derived from human embryonic cultures. The dotted lines indicate a culture split. During these early passages, cell counts were done only at fixed intervals and hence do not necessarily indicate the highest count during each passage. TVL is a fibroblast culture of a 12-week human embryo lung. DHL is a fibroblastic culture of a 14-week human embryo lung. ●, TVL-conditioned media; △, DHL-conditioned media; ○, no conditioned media.

**Table 1** Degree of differentiation of fresh uncultured HL-60 cells and cultured cells grown in the presence or absence of DHL-conditioned medium

	Blasts	Promy- elocytes	Myelo- cytes	Segs* †bands †metas	% Per- oxidase positive	% Sudan black positive	Mono- cytes	Lympho- cytes	Eosin- ophils	Baso- phils	Nucleated RBCs
Fresh HL-60	25	49	3	1	55	NT	6	15	0	0	1
Cultured HL-60											
Day 2+CM	19	69	7	4	97	95	1	0	0	0	0
Day 2-CM	23	65	4	6	98	96	2	0	0	0	0
Day 4+CM	23	62	5	8	NT	NT	2	0	0	0	0
Day 4-CM	28	57	7	5	NT	NT	3	0	0	0	0
Day 8+CM	28	56	7	6	98	96	3	0	0	0	0
Day 8-CM	25	58	8	6	100	97	3	0	0	0	0
Day 10+CM	22	62	8	7	NT	NT	1	0	0	0	0
Day 10-CM	28	57	6	8	NT	NT	1	0	0	0	0

Fresh cells, obtained by leukaphoresis, were smeared and stained with Wright-Giemsa. Cultured cells at passage 9 were seeded in Falcon T30 flasks at  $2.5 \times 10^5$  per ml on day 0 in the presence or absence of CM. On the days after seeding as noted, the cells were mounted by centrifuging 0.5-ml aliquots of the cell suspension at 500 r.p.m. for 5 min in a Shandon-Elliott SCA-0030 cytospin centrifuge. After Wright-Giemsa staining, differential cell counts were performed. Peroxidase and Sudan black stains were performed according to techniques referenced in the text. NT, not tested.

\*Segs, segmented neutrophils; †bands, banded neutrophils; †metas, metamyelocytes.

flasks were supplemented with conditioned media (CM) derived from six different monolayer cultures of various first trimester and early second trimester foetal organs as previously described<sup>6</sup>, while control flasks contained no CM. After three weeks incubation leukocyte growth was noted in flasks containing conditioned media from two different human embryonic fibroblast cultures but not in those without CM or containing other sources of CM (Fig. 1). After splitting the leukocytes and further incubation, cell growth continued only in those flasks containing CM from a single lung embryo culture designated DHL (Fig. 1). After several more weeks, however, the cells continued to grow and differentiate along the myeloid series in the absence of any CM supplements. The cells required foetal calf serum for growth, the optimum concentration being 20% in our culture conditions. When seeded in T30 flasks at  $2.5 \times 10^5$  cells per ml the culture reached a saturation density of  $2.5\text{--}3.0 \times 10^6$  cells per ml in 8–9 d with a doubling time of 55–60 h (Fig. 2).

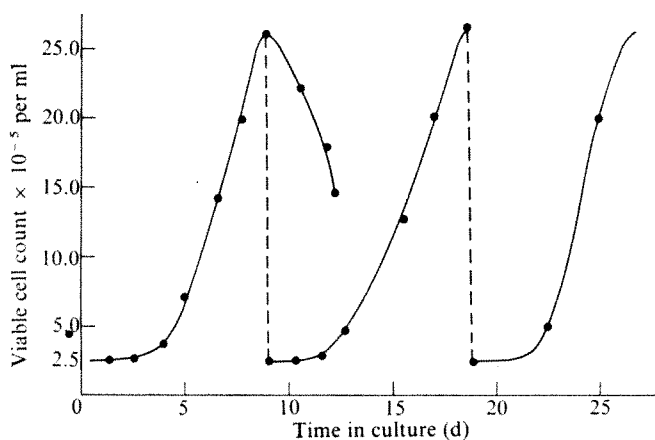
No evidence of mycoplasma contamination as determined by growth assay in agar<sup>7</sup> has been present. Reverse transcriptase assays of the supernatant culture media<sup>8</sup> as well as thin-section electron microscopy of fixed-cell pellets showed no evidence of type-C viral production or expression.

Most of the cells when stained by the Wright-Giemsa procedure were myeloblasts and promyelocytes with azurophilic granules<sup>8</sup>, but more mature myeloid cells (myelocytes, metamyelocytes, bands and segmented neutrophils)

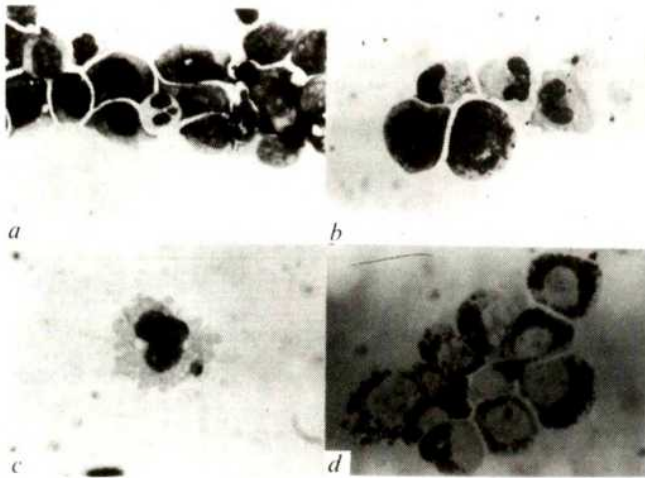
were also seen (Fig. 3a,b and Table 1). A small percentage of the cells resembled monocytes with blunt pseudopods, pale staining cytoplasm, and convoluted nuclei (Fig. 3c and Table 1). Differential counts of the cultured leukocytes remained quite similar throughout the growth phase of the cells (Table 1). Histochemical studies using stains specific for myeloid cells confirmed the myeloid nature of the cell culture with over 95% of the cells staining positive for Naphthol ASD chloroacetate esterase<sup>9</sup>, Sudan black<sup>10</sup>, and peroxidase<sup>11</sup> (Fig. 3d and Table 1). Although the patient's peripheral blood leukocytes from which this culture was derived contained a significant number of lymphocytes (Table 1), no cells resembling lymphocytes are seen in the present culture. In agreement, T- and B-lymphocyte markers as determined by rosette formation with sheep erythrocytes<sup>12</sup>, and complement-bound antibody-coated erythrocytes<sup>13</sup> respectively, were negative. Tests for Epstein-Barr virus nuclear antigen (EBNA)<sup>14</sup> were also negative. Nucleated red blood cells, present in the patient's peripheral blood cells (Table 1), were not seen in the cultured cells and benzidine stains for haemoglobin<sup>15</sup> were negative. Similar morphological and histochemical differentiation has been noted throughout 30 passages of the cell culture over a period of 9 months.

Chromosome studies of the cultured cells (passage 6) revealed aneuploidy with 27 of 40 metaphases examined showing 44 chromosomes and the remaining metaphases varying from 43 to 47 in number. A modal chromosome number of 44 was also observed in short-term cultures of fresh bone marrow obtained from the patient before therapy (J. M. Trujillo, personal communication). These cytogenetic studies together with the morphological similarity between the HL-60 cells and the patient's leukaemic cells strongly suggest that the cells currently being cultured are of leukaemic cell origin. More detailed cytogenetic studies are in progress to confirm this point.

It is unclear exactly what part the conditioned medium played in the origin of the HL-60 cell culture. Attempts to duplicate the experiment using frozen stocks of the patient's fresh uncultured peripheral blood leukocytes were not successful. Also the DHL-conditioned medium failed to stimulate growth of samples of peripheral leukocytes obtained from 18 different myelogenous leukaemia patients, although none of these was of the promyelocytic variant. With the readdition of CM to the growing HL-60 culture, no changes in morphological appearance, histochemical staining, degree of differentiation (Table 1), growth rate, or saturation density were noted. The initial growth of the HL-60 cells only in those flasks containing CM could have

**Fig. 2** Continued growth of HL-60 cells in the absence of conditioned medium. The dotted lines indicate a culture split.





**Fig. 3** Cytological and histochemical characteristics of HL-60 cells cultured in the absence of conditioned medium. *a*, Mature polymorphonuclear leukocyte surrounded by myeloblasts and promyelocytes containing cytoplasmic granules. Wright-Giemsa stain ( $\times 1,000$ ). *b*, Two promyelocytes with prominent cytoplasmic granules together with three metamyelocytes undergoing nuclear differentiation. Wright-Giemsa stain ( $\times 1,000$ ). *c*, Single cell with blunt pseudopods, pale staining cytoplasm, and convoluted nucleus resembling a monocyte. Wright-Giemsa stain ( $\times 1,000$ ). *d*, Positive myeloperoxidase stain which histochemically confirms the myeloid morphological appearance of the cells ( $\times 1,000$ ).

been fortuitous, although further studies are required to assess the interaction of the DHL-conditioned medium with the HL-60 cells as well as with leukaemic cells from other patients with promyelocytic leukaemia.

The presence of a cell culture that clearly differentiates along the myeloid series apparently without the addition of an exogenous source of inducer raises some intriguing questions regarding myelopoiesis in normal and leukaemic cells. It is generally assumed that normal stem cells will differentiate along the myeloid series only when influenced by specific microenvironmental factors, which may be similar to granulopoietin or colony-stimulating factor (CSF)<sup>16</sup>. Previous reports have described differentiation of certain murine and human myeloid leukaemic cells in both suspension<sup>6,17</sup> and agar<sup>18,19</sup>, but only in the presence of appropriate conditioned media or of a 'feeder layer' of peripheral blood leukocytes. Hence the HL-60 myeloid cell culture described here seems to be unique in its ability to proliferate and differentiate without an exogenous source of inducer. These cells may indeed be autonomous with respect to differentiation. It is conceivable, however, that a certain subpopulation of these cultured cells is secreting factors regulating proliferation and differentiation of the remaining cells. Our investigations are directed towards isolating such a subpopulation as well as determining the response of the HL-60 cell culture to the known inducers of myeloid differentiation<sup>20</sup>.

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- Nilsson, K. & Pontén, J. *Int. J. Cancer* **15**, 321-341 (1975).
- Lozzio, C. B. & Lozzio, B. B. *Blood* **45**, 321-334 (1975).
- Klein, E. et al. *Int. J. Cancer* **18**, 421-431 (1976).
- Lozzio, C. B., Lozzio, B. B., Yang, W.-K., Ichiki, A. T. & Bamberger, E. G. *Cancer Res.* **36**, 4657-4662 (1976).
- Barak, Y., Shore, N. A., Higgins, G. R. & Vadakan, V. V. *Br. J. Haemat.* **27**, 543-549 (1974).

- Gallagher, R. E., Salahuddin, S., Hall, W. T., McCredie, K. B. & Gallo, R. C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4137-4141 (1975).
- Hayflick, L. *Texas Rep. Biol. Med.* **23**, 285-303 (1965).
- Bernard, J., Lasneret, J., Chome, J., Levy, J. P. & Boiron, M. *J. clin. Path.* **16**, 319-324 (1963).
- Yam, L. T., Li, C. Y. & Crosby, W. H. *Am. J. clin. Path.* **55**, 283-290 (1971).
- Williams, W. J. in *Hematology* (eds Williams, W. J. et al.) 1395 (McGraw Hill, New York, 1972).
- Williams, W. J. in *Hematology* (eds Williams, W. J. et al.) 1393 (McGraw Hill, New York, 1972).
- Lay, W. H., Mendes, N. F., Bianco, C. & Nussenzweig, V. *Nature* **230**, 531-532 (1971).
- Jondal, M., Holm, G. & Wigzell, H. *J. exp. Med.* **136**, 207-215 (1972).
- Reedman, B. M. & Klein, G. *Int. J. Cancer* **11**, 499-520 (1973).
- LoBue, J., Dornfest, B. S., Gordon, A. S., Hurst, J. & Quastler, H. *Proc. Soc. exp. Biol. Med.* **112**, 1058-1062 (1963).
- Moore, M. A. S. in *Recent Advances in Cancer Research: Cell Biology, Molecular Biology and Tumor Virology* (ed. Gallo, R. C.) (CRC, Cleveland, Ohio, in the press).
- Fibach, E. & Sachs, L. *J. Cell Physiol.* **86**, 221-230 (1975).
- Ichikawa, Y. *J. Cell Physiol.* **74**, 223-234 (1969).
- Moore, M. A. S., Spitzer, G., Williams, N., Metcalf, D. & Buckley, J. *Blood* **44**, 1-18 (1974).
- Krystosek, A. & Sachs, L. *Cell* **9**, 675-684 (1976).

## Non-random chromosome gains in human lymphoblastoid cell lines

HUMAN lymphoblastoid cell lines derived from the peripheral blood lymphocytes of healthy donors are commonly diploid when examined in the early months after establishment but acquire chromosome abnormalities on prolonged culture. Other lines, notably those derived from Burkitt's lymphoma tissue, may display chromosome aberrations from the outset<sup>1-5</sup>. A partial translocation 8q-14q+ has been demonstrated in the majority of Burkitt lymphoma-derived lines<sup>6</sup>, and data on the karyotypes of some human tumours suggest that non-random gains and/or losses of chromosomes may be a feature, in particular, of certain leukaemias and lymphomas<sup>7-13</sup>. As the emergence of an aneuploid clone from a previously diploid lymphoblastoid line may be associated with other changes suggesting the development of a more 'malignant' phenotype<sup>14</sup>, it is relevant to compare the chromosome aberrations detected in such lines with the human tumour data. We have therefore undertaken a study of banded karyotypes of eighty EB virus-carrying human lymphoblastoid lines. The first stage of this analysis is concerned only with gains and losses of whole chromosomes or chromosome arms and the data presented here establish that, considering all the lines together, there have been non-random gains of five autosomes (numbers 3, 7, 8, 9 and 12) and of the sex chromosomes.

Most of the cell lines studied were originally established in this laboratory and all had been maintained in this laboratory for at least 6 months. The derivation of the lines and their 'age' *in vitro* are set out in Table 1. Chromosome analysis was carried out on photographs from quinacrine-stained metaphase spreads<sup>15</sup>. (A more detailed examination of chromosome breaks and translocations in these lines is in progress making use of G and R banded, in addition to quinacrine stained, preparations.) In most instances, serial studies have been made on the lines and the emergence of new chromosomally marked clones has been traced over a period of up to 6 yr<sup>3</sup>. Aberrations detected in only a single cell from a given preparation were ignored while any which recurred in successive clones (identified by additional chromosome markers) from a given line were scored only once for that line. Where a lymphocyte donor had a constitutional chromosome abnormality (Trisomy 21 or XXY, for example) the corresponding abnormality in the derived lymphoblastoid line was not scored as an aberration. There was rarely any difficulty in recognising duplication or deficiency of a whole chromosome or chromosome arm and in establishing that such a marker characterised at least a significant proportion of the cells of a given line. The number of photographs in which a particular aberration could be identified was usually between 6 and 20.

The findings are presented in Fig. 1: 36 lines contribute to these data since eight lines listed as 'aneuploid' in Table 1



Table 1 Lymphoblastoid cell lines studied

Derivation	No. of lines	Age < 1 yr		Age > 1 yr	
		Diploid†	Aneuploid	Diploid	Aneuploid
No lympho-reticular malignancy‡	55	23	2	5	25
Lympho-reticular malignancy§	17	1	0	2	14
Burkitt's lymphoma	8	0	0	0	8
Total	80	24	2	5	49

\**In vitro* 'age' at latest karyotype analysis. Most lines have been followed serially since establishment.

†Status at latest karyotype analysis.

‡Includes lines established from cord bloods, healthy adults, patients with infectious mononucleosis, carriers of constitutional chromosome aberrations (see text), and patients with cancer of bladder or gastrointestinal tract.

§Includes lines from patients with acute and chronic leukaemias, myelofibrosis, and Waldenstrom's macroglobulinaemia. Excludes Burkitt's lymphoma.

had internal rearrangements without substantial gains or losses and a further seven (including three from Burkitt's lymphoma) were near-tetraploid.

It is evident that gains occur much more frequently than losses. In fact apparent absence of a chromosome has always been associated with the presence of one or more unidentifiable abnormal chromosome whose total length is at least equal to that of the material otherwise unaccounted for. The only exception to this rule is the Y chromosome which was lost from two lines. The residual stemline in one is 45,X. The other was derived from a 47,XXY Klinefelter

syndrome patient and the two X chromosomes remain. In the same line there were gains of chromosomes 3, 8, 11 and 12.

Analysis of the total data shows that trisomy or partial trisomy for five autosomes and the sex chromosome pair occurs with remarkable frequency. Out of a total of 66 additional chromosomes or chromosome arms, more than two-thirds (47) are accounted for by only 6 of the 23 pairs, numbers 3, 7, 8, 9, 12 and X+Y (treating the sex chromosomes as one pair). On the null hypothesis that all pairs contribute equally to the additional chromosomes or chromosome arms, it can be shown that the probability that six pairs account for at least two-thirds of them is less than  $10^{-6}$ . No single line is trisomic for all of these chromosomes but in four cases trisomy 12 is associated with an extra number 3, 8 or 9 and in one other line (mentioned above) there is trisomy for three of the five autosomes.

The cultures examined include multiple (two to four) lines derived from the blood lymphocytes of each of seven donors. Given that the distribution of extra chromosomes in the total series of cultures is non-random, there seems to be no particular tendency for identical aberrations to recur in multiple lines from a single donor. Eight other lines were grown from the blood lymphocytes of patients with constitutional chromosome aberrations (45,X, 47,XXY, 49,XXXXY, 47,XYY, trisomy 21 and balanced autosomal translocations). As a group they have shown no greater or lesser tendency to develop further aneuploidy *in vitro* than lines from chromosomally normal donors.

Trisomy 7 has been noted in at least two fresh Burkitt lymphoma biopsies and in previously-described Burkitt lymphoma cell lines<sup>3</sup>. Our data support the view that this aberration has a particular association with Burkitt's lymphoma since trisomy 7 (in one case partial) was found in four of the five Burkitt lines which could be scored, Daudi, Namalva, Jijoye and RAJI. Three other lines, EB<sub>1</sub>, EB<sub>2</sub> and EB<sub>3</sub> are near tetraploid and rather variable in chromosome complement while EB<sub>1</sub> has four large abnormal chromosomes any of which may include a substantial part of an additional number 7. Our results show, however, that trisomy 7 is not restricted to Burkitt-derived lines and the same aberration has also been reported in two lymphosarcoma biopsies, in lymphoblastoid lines from lymphosarcoma and Hodgkin's disease and in cultures of melanoma tissue<sup>6-8</sup>.

Trisomy 8 has been observed with striking frequency in the acute phase of chronic myeloid leukaemia, in acute non-lymphatic leukaemia, in polycythaemia vera and in other myeloproliferative disorders. In all of these conditions except myeloid leukaemia, trisomy 9 also occurs more often than would be expected if extra chromosomes were distributed at random<sup>9-12</sup>. There is thus some suggestion of concordance between specific chromosome gains observed in cultured human lymphoblastoid lines and in malignancy, particularly when it affects the lympho-reticular system. On the other

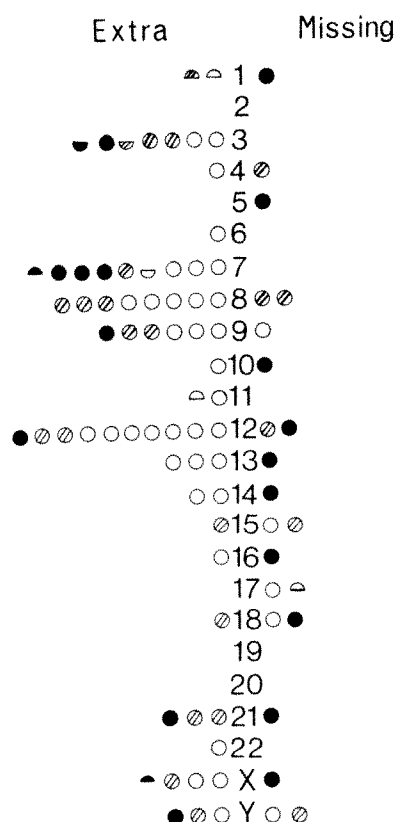


Fig. 1 Cumulative chromosome gains and losses in 36 lymphoblastoid lines. Each line trisomic for a given chromosome is recorded as a full circle to the left of that chromosome number; monosomy is indicated by a full circle to the right. Lines are grouped as in Table 1. Open symbols, lines from patients with no lymphoreticular malignancy; cross-hatched symbols, lines from patients with lymphoreticular malignancy, not Burkitt's lymphoma; filled symbols, Burkitt's lymphoma-derived lines. Partial trisomy or monosomy indicated by upper semicircle (short arm) or lower semicircle (long arm). In addition to gains or losses indicated, there were 19 unidentifiable abnormal chromosomes in the total sample, nine from four Burkitt's lymphoma-derived lines, six among three lines from patients with non-Burkitt's lymphoma lymphoreticular malignancy and one each in four other lines.

hand, trisomy 3 or 12 do not seem to be characteristics of any human neoplasms reported so far and in acute leukaemias loss rather than gain of one chromosome seems to be the norm<sup>11,12</sup>. Furthermore, there are several examples of non-random chromosome gains or losses in particular human tumours which do not correspond to the changes observed in our material<sup>10-13</sup>.

The phenomenon of non-random chromosome gains in cultured human lymphoblastoid lines is established by this study. Its significance may be tested by planned experiments in xenotransplantation involving diploid and aneuploid cells from the same lines.

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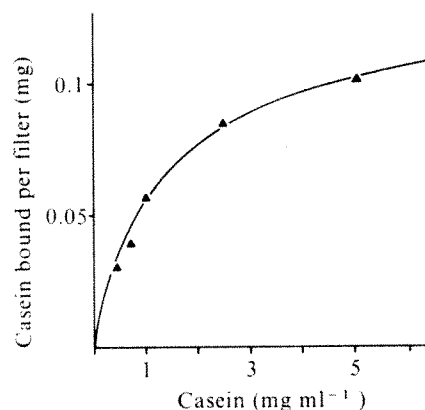
1. Nadkarni, J. S. *et al. Cancer* **23**, 64 (1969).
2. Macek, M. *et al. Cancer Res.* **31**, 308 (1971).
3. Steel, C. M., McBeath, S. E. & O'Riordan, M. L. *J. natn. Cancer Inst.* **47**, 1203 (1971).
4. Bloom, A. D., McNeill, J. A. & Nakamura F. T. in *Chromosomes and Cancer* 565 (ed. German, J.) (Wiley, New York, London, Sydney and Toronto, 1974).
5. Zech, L., Haglund U., Nilsson K. & Klein G. *Int. J. Cancer* **17**, 47 (1976).
6. Manolov, G. & Manolova, Y. *Nature* **237**, 33 (1972).
7. Fleischman, E. W., Hakansson, C. H., Levan, A. & Moller, T. *Hereditas* **70**, 243 (1972).
8. McCulloch, P. B., Dent, O. B., Hayes, P. R. & Liao, S. K. *Cancer Res.* **36**, 398 (1976).
9. Rowley, J. D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 152 (1975).
10. Mitelman, F., Levan G., Nilsson O. G. & Brandt L. *Int. J. Cancer* **18**, 24 (1976).
11. Rowley, J. D. *Cancer* **36**, 1748 (1975).
12. Mitelman, F. & Levan G. *Hereditas* **82**, 167 (1976).
13. Mark, J. *Adv. Cancer Res.* **24**, 165 (1977).
14. Nilsson, K. *Haematology Blood Transfusion* **20**, 253 (1977).
15. Steel, C. M. *Nature* **233**, 555 (1971).

## Essential role of surface-bound chemoattractant in leukocyte migration

MANY chemotactic factors, usually proteins or peptides, have been isolated and studied, but little is known about the basic mechanism of leukocyte migration. This movement is termed chemotaxis if its direction is determined by substances in the cells' environment<sup>1</sup>. The chemotactic agent is assumed to convey information to the leukocytes by interaction with receptors. The subsequent sequence of events thus triggered in the cells is unknown but metabolic changes such as activation of an esterase have been reported as occurring as the cells move forward (for review see ref. 2). A role for surface-bound chemoattractant in cell locomotion was suggested by the observation that mouse fibroblasts move preferentially from substrates of lesser to those of greater capacity to bind to cells<sup>3,4</sup>. A close relationship has also been suggested between the mechanism of chemotaxis and phagocytosis<sup>5</sup>. In both cases cells form protrusions of motile membrane which carry receptors for either chemoattractants or opsonins. For example, such membrane protrusions have been shown to bind bacteria and also to attach to opsonised particles, through Fc receptors to IgG-Fc or through C3 receptors to C3 fragments. Once initial contact is established during phagocytosis further membrane segments bind, enabling the cell to creep around the bacterium until it is ingested. We report here that using casein, a chemotactic agent in a standard chemotaxis assay<sup>6</sup>, we have found that binding of casein to a solid substratum is essential for its attracting capacity.

Two sets of microporous cellulose nitrate filters were prepared. To the first set <sup>125</sup>I-casein (casein, from Merck, Darmstadt)

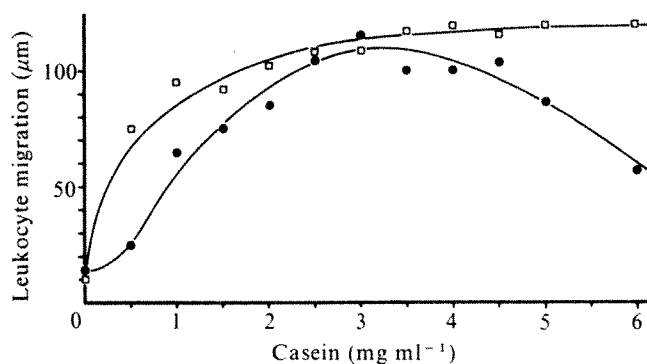
was dissolved in NaOH and adjusted to pH 7.35 by HCl) was bound in a dose-dependent manner (Fig. 1) by incubating the filters for 10 min at room temperature with casein concentrations ranging from 0.5 to 6 mg ml<sup>-1</sup>. Unbound casein was removed by placing the filters in a suction device and washing with 25 ml of Hank's balanced salt solution (HBSS), after which no additional <sup>125</sup>I-casein could be eluted. The second set of filters was treated with HBSS only. To test for surface-bound chemotactic factors, the filters were placed in modified Boyden chambers<sup>7,8</sup>. The upper compartments were filled with a suspension of 2 × 10<sup>6</sup> guinea pig peritoneal granulocytes, which were collected 18–22 h after injection of glycogen (0.1%, w/v), and the lower compartments were filled with either HBSS (casein-treated filters) or increasing amounts of casein (HBSS-treated filters). In the latter case, the casein concentrations in the lower compartments were the same as those used to prepare the casein-treated filter. After incubation for 90 min at 37 °C the distance migrated by the granulocytes into the filter was measured.



**Fig. 1** Uptake of casein by cellulose nitrate filters. Cellulose nitrate filters (3 µm pore size, 25 mm diameter, type AE97, Schleicher & Schüll) were incubated (10 min at room temperature) in 2 ml of solutions containing increasing concentrations of <sup>125</sup>I-labelled casein. The filters were then placed in a suction device (type FN 023/0, Schleicher & Schüll) and 25 ml of HBSS were passed through the filters. Based on the amount of radioactivity bound per filter, the total amount of casein attached to the filter was determined.

With casein concentrations of approximately 3 mg ml<sup>-1</sup>, almost identical migration distances were obtained (Fig. 2) whether the filters were preincubated in casein solutions (first set) and used without further addition of casein to the Boyden chamber, or whether the filters were initially free of casein (second set) and used in the test by adding casein to the lower compartment of the chamber. At casein concentrations of 0.5 and 1 mg ml<sup>-1</sup> the granulocytes in the first set of filters were found to have migrated slightly longer distances than the granulocytes in the second set of filters. Concentrations of casein higher than 3 mg ml<sup>-1</sup> led to opposite results for the two sets of filters. While with the first set of filters a slight increase in migration distance was observed (Fig. 2), the second set yielded a continuous decrease of the migration distance in parallel with the increase in casein concentration in the lower compartment. This reduction distance can therefore only be attributed to the presence of casein which was not bound to the substratum. These experiments were repeated 10 times and showed a clear reproducibility. If purified casein was used for the experiments instead of the commercially available casein, similar results were obtained with filter-bound casein whereas in the standard chemotactic assay higher casein concentrations were required to affect inhibition of migration (data not shown).

To confirm the role of surface-bound chemoattractant, filters soaked with a solution of 2.5 mg <sup>125</sup>I-casein per ml and washed in HBSS, were incubated for 20 min at 37 °C with solutions of



**Fig. 2** Effect of filter-bound casein on leukocyte migration. Cellulose nitrate filters prepared as described in the legend to Fig. 1 were used in the Boyden chamber with 1 ml of  $2 \times 10^6$  guinea pig peritoneal granulocytes in the upper compartment and HBSS in the lower compartment ( $\square$ ). For controls, filters preincubated in HBSS instead of casein were used with solutions of increasing casein concentration in the lower compartment ( $\bullet$ ). Along the abscissa the casein concentrations are indicated which, in the case of the first set of filters ( $\square$ ), were present during the preincubation and, in the case of the control filters ( $\bullet$ ), during the actual migration test.

increasing concentrations of papain ( $1 \times 10^{-8}$ – $1 \text{ mg ml}^{-1}$ ) and the papain-induced release of casein from the filter was followed (Fig. 3). It was found that increasing concentrations of papain led to increasing release of radioactive material and, simultaneously to decreasing migration distances. In control experiments it was shown that preincubation of the filter with papain, followed by washing with HBSS had no effect on the migration of the leukocytes towards the casein in the lower compartment. Thus we could exclude the possibility that any papain remaining in the filters after the washing procedure would interfere with migration of the cells.

These results suggest that filter bound casein has the essential role in the promotion of casein-induced leukocyte migration, and that casein in free solution may even be inhibitory. Whether this is due to receptor blockade or to toxic effects is not clear. From our experiments with purified casein, the latter possibility seems to be more likely. It is also important to establish whether other chemoattractants behave like casein. If this is a general characteristic, cells responding to a chemotactic stimulus could be envisaged as creeping along by holding on to surface-bound chemoattractants with special receptors or receptive

sites. Binding of fluid phase reagents to the same cell receptors might result in blocking the interaction of these receptors with the surface-bound material. Also, fluid-phase reagents might bind first to the cell receptors, and thereby promote secondary binding to a nearby surface, or they might stimulate cells to secrete factors with functional characteristics similar to those of casein. This is not so far fetched, as we have observed that supernatants obtained by incubating leukocytes for 30 min at  $37^\circ\text{C}$  in HBSS contain substances which, like casein, modulate filters so that they now can induce the locomotion.

According to this view, leukocytes migrating *in vivo* would be crawling along tissue surfaces coated with chemoattractants. By analogy with fibroblasts<sup>3,4</sup> the cells would migrate to where there was a greater amount of attractive substances fixed per unit surface area which would coincide with a tighter binding. This is supported by preliminary experiments with filters to which casein was not attached uniformly but with an increasing concentration from one side to the other (unpublished results). This movement would only be possible if unoccupied receptors could be made available continuously at the leading edge of the cell or on the protrusions actively reaching out from the cell body searching for sites of attachment. Therefore the cells must either be able to synthesise new receptor sites or to re-use the already existing ones. Harris's concept<sup>9</sup> that the surface proteins in moving cells continuously circulate within the membrane from the front of the cell to its trailing end, then become internalised and, after a hypothetical passage through the cytoplasm, become incorporated again into the front section of the cell, seems to be most attractive. It is possible that proteolytic activity might be involved in freeing the receptors from the bound substance, since chemotactic factors are known to induce release of lysosomal enzymes<sup>10–12</sup>.

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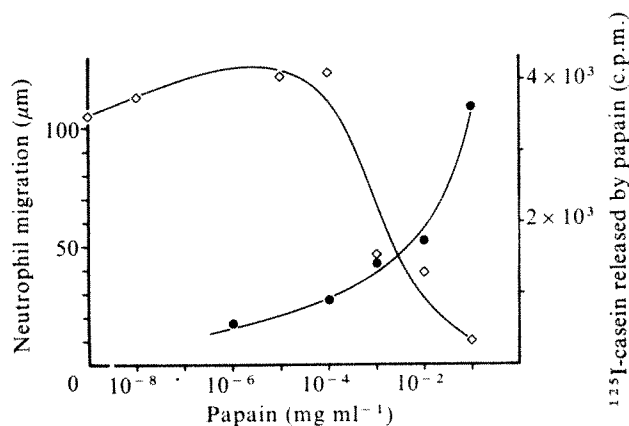
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- Keller, H. U. *et al. Clin. exp. Immun.* **27**, 377–380 (1977).
- Wilkinson, P. C. *Chemotaxis and Inflammation* (Churchill and Livingstone, Edinburgh and London, 1974).
- Carter, S. B. *Nature* **208**, 1183–1187 (1965).
- Harris, A. *Expl Cell Res.* **77**, 285–297 (1973).
- Griffin, F. M., Griffin, J. A., Leider, J. E. & Silverstein, S. G. *J. exp. Med.* **142**, 1263–1282 (1975).
- Keller, H. U. & Sorkin, E. *Int. Arch. Allergy appl. Immun.* **31**, 575 (1967).
- Boyden, S. V. *J. exp. Med.* **115**, 453 (1962).
- Zigmond, S. H. & Hirsch, J. G. *J. exp. Med.* **137**, 387 (1973).
- Harris, A. K. *Nature* **263**, 781–783 (1976).
- Goldstein, I., Hoffstein, S., Gallin, J. & Weissmann, G. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2916 (1973).
- Becker, E. L., Showell, H. J., Henson, P. M. & Hsu, I. S. *J. Immun.* **112**, 2047–2054 (1974).
- Showell, H. J. *et al. J. exp. Med.* **143**, 1154 (1976).



**Fig. 3** Reduction of casein-dependent leukocyte migration by pretreatment of the  $^{125}\text{I}$ -casein-coated filters with papain. Filters incubated in a solution of  $2.5 \text{ mg } ^{125}\text{I}$ -casein per ml and washed in HBSS were incubated 20 min at  $37^\circ\text{C}$  with solutions of increasing concentrations of papain (abscissa) containing appropriate amounts of cysteine. Then the filters were washed and the release of radioactivity was determined ( $\bullet$ ). The same filters were tested for leukocyte migration ( $\diamond$ ).

## Membrane-bound H-2 and H-Y antigens move independently of each other

ON exposure to foreign antigens, mouse T lymphocytes can be stimulated to mature into cytotoxic cells able to kill target cells carrying these antigens<sup>1–4</sup>. Killing is restricted to cells bearing, in addition to the foreign antigen, the same H-2K or H-2D antigens as the stimulating cell. Cells bearing different H-2 antigens are not killed. To kill a lymphocyte, two recognition steps are necessary: one involving the foreign antigen and another involving H-2

antigens. This associative recognition has been demonstrated for many antigens and must be considered a general phenomenon. Two opposing views have been proposed to explain the associative recognition<sup>1</sup>. According to the altered-self hypothesis, the antigen links up on the cell membrane with H-2 molecules and the complex of antigen plus H-2 is then recognised by a single receptor on a T lymphocyte. According to the dual recognition hypothesis, each T lymphocyte has two receptors, one for the antigen and the other for H-2, and it is the combination of the two receptors on each cell that is responsible for the associative recognition. The strongest evidence in favour of the first hypothesis is the report of a physical association between antigens and H-2 (see, for example, ref. 6). The link-up of the antigen to H-2, however, might not be related directly to the associative recognition, but rather reflect some other phenomenon in the membrane, in which case there should be examples of associative recognition without a linkage between the antigens. We present evidence for such an association.

Associative recognition has been demonstrated for H-Y, the male-specific antigen. Gordon *et al.*<sup>3</sup> showed that C57BL/10 female mice, when primed to H-Y, develop cytotoxic effector cells which are able to lyse male target cells carrying the same H-2 haplotype as the females (H-2<sup>b</sup>). According to the altered-self hypothesis, the H-Y and H-2 should form supramolecular complexes that, in the membrane, redistribute together when exposed to either H-Y or H-2 antibody. We tested this assumption as follows. We incubated C57BL/6 (H-2<sup>b</sup>) male thymocytes at 37 °C with either anti-H-2K.33 or anti-H-2D.2 (or both sera) which react with private antigens controlled by the H-2K<sup>b</sup> and H-2D<sup>b</sup> alleles of the H-2<sup>b</sup> haplotype, respectively. We then stained the cells with rabbit anti-mouse IgG (RAMIG) serum conjugated with tetramethylrhodamine isothiocyanate (TRITC)<sup>7</sup>. In these conditions most of the H-2 molecules were in a cap at one pole of the cell. We then applied to these capped cells the mouse anti-H-Y serum at 0 °C and

determined the distribution of the H-Y antibodies using goat anti-mouse IgG (GAMIG) conjugated with fluorescein isothiocyanate (FITC). If H-2 and H-Y were associated, then capping of H-2 should cause capping of H-Y. In this case, no H-Y should remain outside the H-2 caps (the red and green fluorescence of TRITC and FITC, respectively, should overlap and be seen only in the cap with the remainder of the cell not staining at all). If, on the other hand, H-2 and H-Y were not physically associated, there should be a red cap of H-2 and diffuse green staining of H-Y (and—in cases where the GAMIG antibodies find places on the cap unoccupied by the RAMIG—possibly also green cap).

The results are shown in Table 1. When B6 cells were first incubated at a capping temperature (37 °C) with anti-H-2K.33 serum, about one-half stained specifically for the H-2K.33 antigen and in all of them the red TRITC stain had gathered in caps. (The thymocyte population is heterogeneous in terms of the degree of H-2 antigen expression; the weakly H-2 positive cells were classified as negative in the staining conditions used in this experiment.) When these cells were re-exposed to the same antiserum (anti-H-2K.33) in non-capping conditions, no additional staining could be picked up by the FITC-GAMIG antiserum. On exposure to anti-H-2D.2 or to anti-H-Y serum, however, 50 and 60% of the cells, respectively, picked up diffuse green fluorescence. These percentages closely correspond to the percentages of cells reacting with the two antisera in a situation where anti-H-2K.33 was replaced by a medium. We conclude, therefore, that capping of H-2K.33 did not result in any significant capping of H-2D.2 or H-Y. Similar results were obtained when the anti-H-2D.2 serum was used first and the anti-H-2K.33 and anti-H-Y sera second. In this instance the three antigens again redistributed in the cell membrane independently of one another. When the cells were incubated at 37 °C with a mixture of anti-H-2K.33 and anti-H-2D.2 sera, virtually no cells with diffuse green fluorescence were found after re-exposure at

**Table 1** Redistribution of H-2 and H-Y antigens after treatment of C57BL/6J male thymocytes with corresponding antisera

First treatment (37 °C)	Second treatment (0° to 4 °C)	No. of cells counted	% Of cells stained with TRITC Redistributed	% Of cells stained with TRITC Diffuse	% Of cells stained with FITC Redistributed	% Of cells stained with FITC Diffuse + caps
Anti-H-2K.33 + TRITC-RAMIG	Anti-H-2K.33	208	100 (48)	0	100 (48)	10 (5)
	+ FITC-GAMIG*					
	Anti-H-2D.2	222	100 (45)	0	24 (11)	111 (50)
	Anti-H-Y	312	100 (32)	0	31 (10)	186 (60)
Anti-H-2D.2 + TRITC-RAMIG	Medium	250	100 (40)	0	100 (40)	15 (6)
	Anti-H-2D.2	200	100 (50)	0	98 (49)	8 (4)
	Anti-H-2K.33	200	104 (52)	0	22 (11)	116 (58)
	Anti-H-Y	230	99 (43)	0	51 (22)	127 (55)
	Medium	208	100 (48)	0	100 (48)	8 (4)
Anti-H-2K.33 + Anti-H-2D.2 + TRITC-RAMIG	Anti-H-2K.33	185	100 (54)	0	100 (54)	11 (6)
	Anti-H-2D.2	160	100 (63)	0	100 (63)	18 (11)
	Anti-H-Y	138	100 (72)	0	41 (30)	94 (68)
	Medium	200	100 (50)	0	106 (53)	8 (4)
Medium	Anti-H-2K.33	100	—	—	9 (9)	77 (77)
	Anti-H-2D.2	100	—	—	12 (12)	79 (79)
	Anti-H-Y	109	—	—	5 (6)	57 (52)
	Medium	100	—	—	2 (2)	0 (0)

Thymuses were removed without parathymic lymph nodes. Cell suspension was prepared by pressing the organ through a stainless-steel sieve (50-mesh) into Eagle's essential medium (GIBCO) containing 20% heat-inactivated foetal calf serum and cycloheximide (100 µg ml<sup>-1</sup>, pH 7.4–7.6 (hereafter referred to as 'medium')). Cells were washed twice and resuspended in the medium. For the first treatment, aliquots of thymocyte suspension containing 5 × 10<sup>6</sup> cells were distributed into small tubes and centrifuged. Alloantiserum or medium, 50 µl, was added to the packed cells, resuspended cells were incubated for another 30 min at 37 °C. After one washing, incubations with alloantiserum (medium) and TRITC-RAMIG were repeated once more. For the second treatment, the cell suspensions were divided into four tubes and transferred to an ice bath. The packed cells were incubated for 30 min with alloantiserum or medium, and, after one washing, with FITC-GAMIG for 30 min. After the last incubation, cells were washed three times at 0° with medium and fixed with 1% paraformaldehyde in phosphate buffered saline. A drop of the cell suspension was placed on microscope slide, allowed to dry, and mounted in 90% glycerol. The slides were scored using a Leitz Ortholux II fluorescence microscope equipped with a Ploem epi-illumination system. Antisera were as follows. Anti-H-2K.33: (B10.D2 × A)F<sub>1</sub> anti-B10. A(5R); anti-H-2D.2: [C3H/HeJ × B10.D2(R107)]F<sub>1</sub> anti-B10. A(4R); anti-H-Y: C57BL/6J anti-C57BL/6J. The H-2 sera were produced as described elsewhere<sup>8</sup>; anti-H-Y serum was prepared by inoculating C57BL/6 females every 2 weeks with 25 × 10<sup>6</sup> C57BL/6J male spleen cells i.p., and the antiserum was obtained 10 d after the seventh inoculation. The activity of H-2 and H-Y antisera was determined by cytotoxic test against lymphocytes<sup>9</sup> and spermatozoa<sup>10</sup>, respectively.

\*In all 'second treatments' cells were incubated with FITC-GAMIG after initial incubation with alloantiserum or medium.



0 °C to either of the two antisera separately: all the detectable H-2 antigens were capped. But the cells still stained diffusely after exposure to H-Y antiserum. The percentage of the cells with green diffuse fluorescence was once again comparable to that in the aliquots treated first with medium and then with anti-H-Y serum. We conclude, therefore, that complete capping of H-2 antigens has no detectable effect on the distribution of the H-Y antigen: the two types of antigen apparently are not linked in the cell membrane. This conclusion, however, requires at least two qualifications. First, it is possible that while the majority of H-Y molecules are not associated with H-2, a small number are; the latter might not be detected by our method. Second, H-2 and H-Y molecules might be associated before the exposure to antibodies and the antibody might break such bonds. Assuming that neither of these two qualifications applies, one has to conclude either that the H-Y associative recognition is a special case and its mechanism is different from associative recognition of other antigens, or that linkage between the antigen and H-2 is not required for the recognition to occur. Since we find little evidence to support the former possibility, we favour the latter. Our finding does not, however, resolve the dispute between altered-self and dual recognition. It is possible, for instance, that what is needed for recognition to occur via a single receptor is that the H-2 and H-Y (or other antigens) come close to each other in the membrane. With the membrane being in a semifluid state, there is ample opportunity for such an event to occur.

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1. Zinkernagel, R. H. & Doherty, P. C. *Nature* **248**, 701–702 (1974).
2. Bevan, M. J. *J. exp. Med.* **142**, 1349–1364 (1975).
3. Gordon, R. D., Simpson, E. & Samelson, L. E. *J. exp. Med.* **142**, 1108–1120 (1975).
4. Shearer, G. N. *Eur. J. Immun.* **4**, 527–533 (1974).
5. Zinkernagel, R. H. & Doherty, P. C. *Nature* **241**, 1427–1437 (1975).
6. Schrader, J. W., Cunningham, B. A. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5066 (1975).
7. Geib, R., Poulik, M. D., Vitetta, E. S., Kearny, J. F. & Klein, J. *J. Immun.* **117**, 1532–1537 (1976).
8. Klein, J., Klein, D. & Shreffler, D. C. *Transplantation* **10**, 309–320 (1971).
9. Klein, J., Hauptfeld, V. & Hauptfeld, M. *Immunogenetics* **2**, 141–150 (1975).
10. Goldberg, E. H., Boyse, E. A., Bennett, D., Scheid, M. & Carswell, E. A. *Nature* **232**, 478–480 (1971).

## Rate of offset of action of slow-acting muscarinic antagonists is fast

ATROPINE generally behaves as a competitive antagonist of acetylcholine (ACh) at muscarinic receptors<sup>1–9</sup> but its onset and offset of action are slow. It has been suggested that the rates of onset and offset of atropine blockade are determined by the rates of binding to and dissociation from the ACh receptor<sup>4,5,10</sup> a suggestion which at first sight seems incompatible with competitive blockade. An alternative explanation is that access of antagonist (in contrast to agonist) to the receptors, and escape from their vicinity on washing, is delayed or impeded in some

way<sup>11–15</sup>. Experiments in which atropine is applied to a small superficial region of the tissue would seem to offer the best chance of reducing this access factor to a minimum and help in deciding which explanation for atropine's slow action is correct. The iontophoretic method presents itself as the method of choice, but until now, no such experiments have been carried out. The results of this type of experiment carried out on smooth muscle are described here. They reveal that the rate of offset of blockade by slow acting muscarinic antagonists can be more than ten times faster than that seen when antagonists are applied by their addition to the solution bathing (or perfusing) the muscle.

The iontophoretic application of ACh (typically using a 50-nA, 2.5-nC pulse) usually elicited a monophasic depolarisation with a latency of 0.1–1 s (Fig. 1) although bi- and polyphasic responses were also observed<sup>18</sup>. The sensitivity to ACh was tested every 16–20 s, or occasionally every 8 s, and in stable preparations the response varied little. Three muscarinic blocking agents were studied for their effects on the ACh (or carbachol) response. These were: atropine sulphate, (2-benzilyloxyethyl) diethylmethyl ammonium iodide (the diethyl analogue of lachesine<sup>19</sup>, DE lach) and the partial agonist hexyl trimethyl ammonium iodide (hexyl TMA)<sup>2,14</sup>. DE lach resembles lachesine except that one of the quaternary nitrogen methyl groups is replaced by an ethyl group. This marginally increases its potency<sup>19</sup>. The release of any one of these substances from the other barrel of a double-barrelled iontophoretic pipette could reduce or abolish the response to ACh or other muscarinic agonist (Fig. 1). If no further blocking agent was applied, the responses recovered their former size at a rate which was different for each drug.

**Table 1** Rate of offset of blockade by muscarinic antagonists

Antagonist	No. of experiments	Time (s)*		$K_{off}(s^{-1})$
		Half recovery	Full recovery	
Atropine	14	62 ± 15	216 ± 24	~ 11 × 10 <sup>-3</sup>
DE lach	10	21 ± 1.6	46 ± 2.2	~ 34 × 10 <sup>-3</sup>
Hexyl TMA	1	9	25	~ 77 × 10 <sup>-3</sup>

\*Mean ± s.e.m.

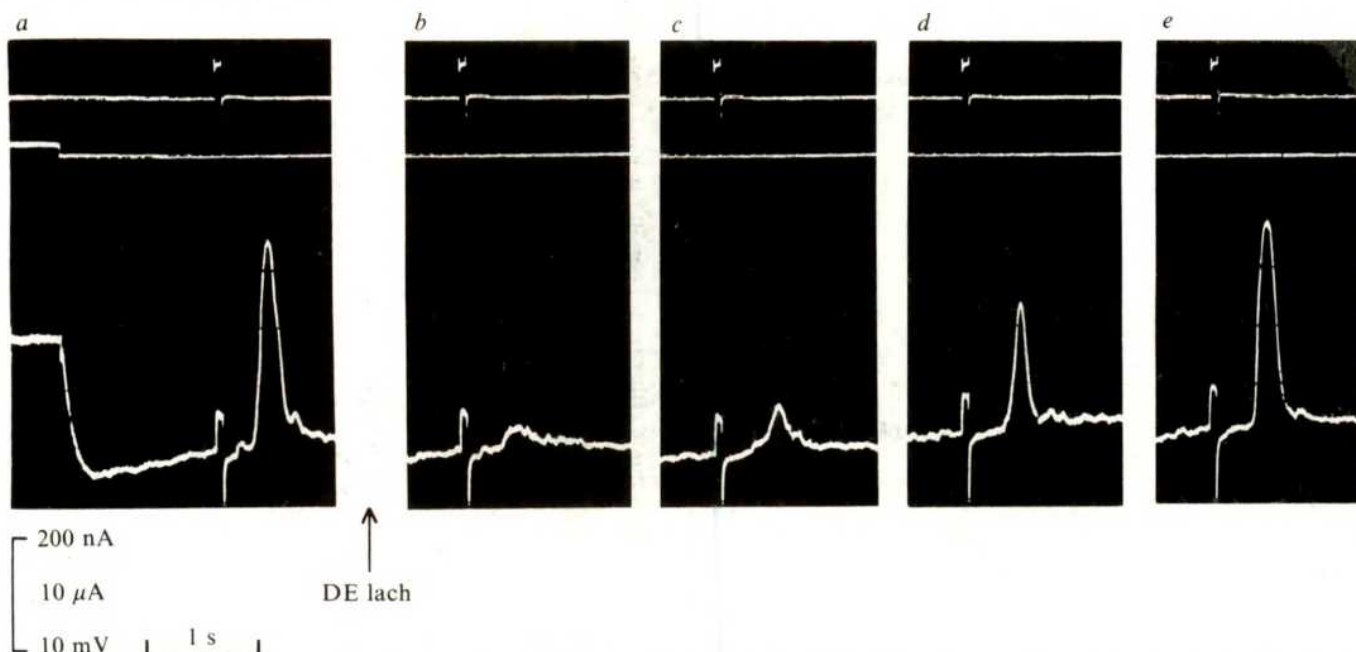
Pulses of antagonist were chosen which produced initially 90–100% reduction in the response to ACh or other agonist. To obtain a quantitative measure of the speed of offset of antagonism, the times from first re-appearance of the response to 50% recovery or to full recovery, were measured in a number of experiments (Table 1). The effects of an atropine or DE lach pulse sufficient initially to nearly or completely abolish the response to ACh were completely absent on average after about 3.5 min and 45 s,

**Table 2** Summary of rate constants of offset of antagonist action as measured by various workers

Antagonist	$K_{off}(s^{-1})$	Tissue	Reference
Atropine	0.3 × 10 <sup>-3</sup>	Atria	12
	1.3 × 10 <sup>-3</sup>	Perfused heart	12
	1.8 × 10 <sup>-3</sup>	Longitudinal muscle ileum	5
	0.3 × 10 <sup>-3</sup>	Longitudinal muscle ileum	4
	0.9 × 10 <sup>-3</sup>	Longitudinal muscle ileum	13
Lachesine	11 × 10 <sup>-3</sup>	Taenia	This work
	3.7 × 10 <sup>-3</sup>	Longitudinal muscle ileum	5
	0.7 × 10 <sup>-3</sup>	Longitudinal muscle ileum	13
DE lach	34 × 10 <sup>-3</sup>	Taenia	This work

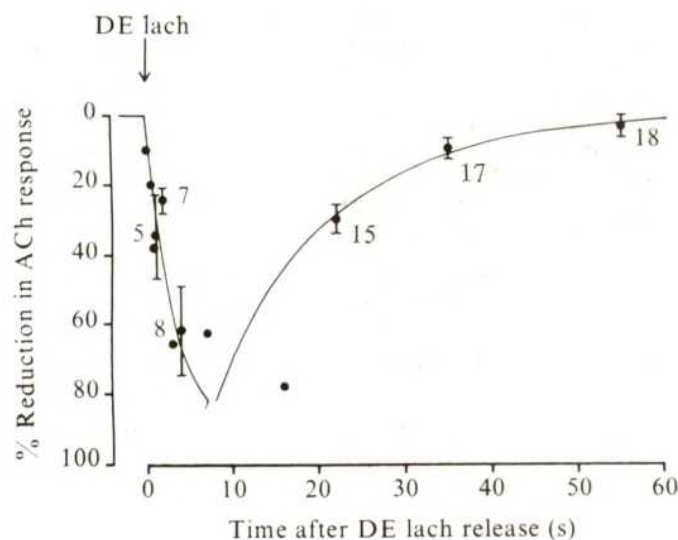
respectively, and recovery to half size was achieved at about 1 min and about 20 s.

To facilitate comparison with the work of others, the time to half-recovery has been multiplied by the factor 1.44 to obtain the rate constant of offset of antagonism ( $K_{off}$ ), assuming an exponential decline of occupancy by blocking agent and a linear relationship between response and agonist dose<sup>12</sup>. It is doubtful if these assumptions are strictly justified but the error involved is insignificant when compared with the rates of offset of blockade



**Fig. 1** Block of response to iontophoretic ACh by an iontophoretic pulse of DE lach and its subsequent recovery. *a*, Lowest trace (10 mV calibration). Depolarisation caused by the stimulation of the muscarinic receptors of smooth muscle by ACh (3.6 nC) released as indicated by the top iontophoretic monitor trace (200 nA calibration). Membrane potential was recorded intracellularly by microelectrode within 25  $\mu$ m of the point of application of ACh from one barrel of a twin-barrelled iontophoretic pipette with spacer<sup>16</sup>. The membrane potential before each application of ACh was first hyperpolarised by passing inward current (middle trace and 10  $\mu$ A calibration) to prevent spiking and contraction. This part of the record is shown in *a* but is omitted in subsequent ones. Later responses to ACh were obtained at 8 s, *b*; 24 s, *c*; 40 s, *d*; and 56 s, *e* after DE lach (8 nC, not shown) which was applied from the other barrel of the iontophoretic pipette. The preparation was a narrow longitudinal strip cut from the longitudinal muscle of the guinea pig caecum (taenia). The region of active muscle was further reduced by creating a 'node' of active muscle, perfused with isotonic physiological salt solution (composition given in ref. 17) at 35  $^{\circ}$ C, between two streams of de-ionised sucrose solution in a type of double sucrose-gap apparatus described previously<sup>17</sup>.

given in the literature (Table 2), using muscle preparations bathed or perfused with solution containing the blocking agent. In general, previous measurements of the rate of offset of blockade are at least ten times slower (range 6–49) which argues strongly that in experiments described by others it is factors other than just dissociation from the receptors, which determines the rate of offset of blockade.



**Fig. 2** Time course of onset and offset of blockade by DE lach pulse (16 nC) constructed by combining measurements of the percentage reduction in ACh responses by some 39 separate DE lach pulses placed with varying time relationships to the ACh pulses. Standard errors ( $\pm$ ) are shown only for those points which are the mean of measurements made on at least five responses. The lines (fitted by eye) are exponentials ( $\tau = 3.7$  and 13 s). Notice that maximum block (about 85%) by DE lach occurs some 5–10 s after release of DE lach from the pipette.

The rate of offset of blockade by hexyl TMA was estimated by means of a series of applications of hexyl TMA placed in varying time relationships to agonist responses elicited every 20 s, and the percentage reductions in agonist response measured. Combination of the results obtained with 17 separate applications of hexyl TMA in one experiment enabled it to be estimated that full recovery took place within about 25 s, and half-recovery within 9 s of application of a blocking pulse of hexyl TMA. The blocking action of hexyl TMA could only be demonstrated with large pulses (50–100 nC). Smaller pulses (10–20 nC) were observed to have only agonist action.

The simultaneous release of blocking doses of hexyl TMA and a strong agonist abolished the response to agonist immediately. This was not the case with DE lach. Figure 2 shows the time course of onset of DE lach blockade calculated from the results of 39 separate DE lach applications to one preparation where the timing of blocking pulses of DE lach was varied with respect to regular ACh pulses. Blockade by DE lach took some 5–10 s to become maximal and then offset of blockade commenced. The onset of blockade by atropine could not be studied in detail because it was generally found necessary to pass iontophoretic current for several seconds to release sufficient atropine to produce blockade.

The much greater rate of offset of antagonism by atropine and DE lach seen in the present experiments suggests that the muscarinic ACh receptors involved in the depolarising response may be relatively superficially placed, so that escape of antagonist from the immediate vicinity of the receptors does not determine the rate of offset of blockade. In such a case, the rates of offset of antagonism ( $K_{off}$ ) seen here give lower limits for the dissociation rate constants ( $k_2$ ) for the binding of atropine, DE lach, and hexyl TMA of  $11 \times 10^{-3}$ ,  $34 \times 10^{-3}$ , and  $77 \times 10^{-3} \text{ s}^{-1}$  (Table 1). If it is assumed that the equilibrium constants ( $k_2/k_1$ ) for atropine and DE lach are  $1.1 \times 10^{-9}$  and  $1.4 \times 10^{-9} \text{ M}$  (refs. 4, 5 and 13) then the association rate constants ( $k_1$ ) will be  $10^7$  and  $2.4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  respectively. These association rate constants are approaching the theoretical maximum for enzyme-substrate reactions of about  $10^8 \text{ M}^{-1} \text{ s}^{-1}$  (ref. 21). This supports the hypothesis that the rate of offset of antagonism observed in these experiments may be

giving for the first time a true indication of the rate of dissociation of antagonist from the muscarinic receptor.

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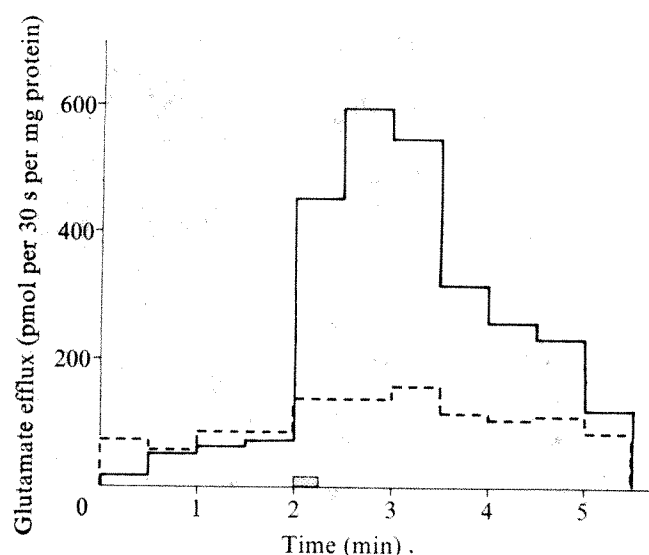
- Clark, A. J. *J. Physiol., Lond.* **61**, 547–556 (1926).
- Stephenson, R. P. *Br. J. Pharmac.* **11**, 379–393 (1956).
- Arunlakshana & Schild, H. O. *Br. J. Pharmac.* **14**, 48–58 (1959).
- Paton, W. D. M. *Proc. R. Soc. Lond.* **B154**, 21–69 (1961).
- Paton, W. D. M. & Rang, H. P. *Proc. R. Soc. Lond.* **B163**, 1–44 (1965).
- Waud, D. R. *Pharmac. Rev.* **20**, 49–88 (1968).
- Pauling, P. J. & Petcher, T. J. *Nature* **228**, 673–674 (1970).
- Rang, H. P. *Nature* **231**, 91–96 (1971).
- Baker, R. W., Chothia, C. H., Pauling, P. & Petcher, T. J. *Nature* **230**, 439–445 (1971).
- Rang, H. P. *Proc. R. Soc. Lond.* **B164**, 488–510 (1966).
- Furchgott, R. F. *Pharmac. Rev.* **7**, 183–265 (1955).
- Thron, C. D. & Waud, D. R. *J. Pharmac. exp. Ther.* **160**, 91–105 (1968).
- Roberts, F. & Stephenson, R. P. *Br. J. Pharmac.* **58**, 57–70 (1976).
- Ginsborg, B. L. & Stephenson, R. P. *Br. J. Pharmac.* **51**, 287–300 (1974).
- Colquhoun, D., Henderson, D. & Ritchie, J. M. *J. Physiol., Lond.* **227**, 95–126 (1972).
- Del Castillo, J. & Katz, B. *Proc. R. Soc. Lond.* **B146**, 339–356 (1957).
- Bolton, T. B. *J. Physiol., Lond.* **250**, 175–202 (1975).
- Bolton, T. B. *Proc. R. Soc. Lond.* **B194**, 99–119 (1976).
- Barlow, R. B., Scott, K. A. & Stephenson, R. P. *Br. J. Pharmac.* **21**, 509–522 (1963).
- Waud, D. R. *J. Pharmac. exp. Ther.* **158**, 99–114 (1967).
- Gutfreund, H. *Enzymes: Physical Principles* (Wiley, London 1972).

## Glutamate as transmitter of hippocampal perforant path

CERTAIN experimental manifestations of plasticity, such as habituation<sup>1–3</sup> and long-term potentiation<sup>2,4–6</sup>, can be readily demonstrated at the perforant path-granule cell synapse in the fascia dentata, and these adaptations are believed to have behavioural counterparts. The perforant path-granule cell synapse may thus serve as a model for investigating the simplest components of behaviour and intellectual function. Detailed studies would, however, require knowledge of the transmitter used by the perforant path fibres. Quite apart from the problem of synaptic plasticity, identification of the perforant path transmitter would add substantially to our knowledge of excitatory transmitters in the central nervous system. Here we present pre- and postsynaptic evidence that these fibres use glutamate as their transmitter.

We have previously shown that endogenous glutamate is released by dentate slices<sup>7</sup> and hippocampal synaptosomes<sup>8</sup> in a manner characteristic of transmitter substances and that this release originates in part from the perforant path fibres<sup>9</sup>. These fibres do not release aspartate<sup>9</sup> or  $\gamma$ -aminobutyrate (GABA)<sup>10</sup>. There was also a suggestion<sup>9</sup>, since confirmed<sup>11</sup>, that the perforant path boutons transport glutamate by a high affinity process. While these observations show that glutamate may be the transmitter at the perforant path-granule cell synapse, one could object that the slices and synaptosomes were depolarised only by chemical, and therefore non-physiological, stimuli and that no evidence has been presented implicating glutamate in the postsynaptic response. We have now shown that electrical field stimulation releases endogenous glutamate from slices of the fascia dentata and that a glutamate antagonist reduces the efficacy of perforant path stimulation.

In eight experiments a longitudinal slice of the internal leaf of the rabbit fascia dentata was electrically stimulated while being continuously superfused. When the slice was superfused with a physiological medium containing 2 mM free  $\text{Ca}^{2+}$ , electrical stimulation elevated the rate of glutamate release by a maximum of 300–1,200 pmol per 30 s per mg protein. In three experiments one slice was superfused with normal medium and another with  $\text{Ca}^{2+}$ -free medium supplemented with  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$  to avoid membrane destabilisation (Fig. 1). Superfusion with a  $\text{Ca}^{2+}$ -free medium reduced glutamate output in response to stimulation by 62, 78 and 90%. Thus electrical stimulation, like elevated  $\text{K}^+$  and veratridine<sup>7</sup>,



**Fig. 1** Time course of glutamate efflux from a slice of fascia dentata in the presence and absence of  $\text{Ca}^{2+}$ . In each experiment a single 0.35-mm thick longitudinal slice of the free edge (internal leaf) of the fascia dentata was cut from a dissected rabbit hippocampus and immersed in a physiological medium at 37 °C for 5–10 min. Then the slice was superfused with the same 37 °C-medium at 1 ml min<sup>-1</sup> in a 0.5-ml chamber equipped with spiral silver electrodes. During the period indicated by the dotted bar the tissue was stimulated at 100 Hz with 10 V square wave pulses of alternating polarity and 5 ms duration. Superfusate fractions were collected continuously, and their glutamate content was determined by a micro-enzymatic fluorometric method<sup>18</sup>. The figure shows results from one set of paired experiments. Solid lines, glutamate efflux into normal medium (2 mM free  $\text{Ca}^{2+}$ ); dashed lines, glutamate efflux into  $\text{Ca}^{2+}$  free medium (with 25 mM  $\text{MgCl}_2$  and 0.1 mM  $\text{MnCl}_2$ ).

releases glutamate from the fascia dentata in a  $\text{Ca}^{2+}$ -dependent manner. Since extracellular  $\text{Ca}^{2+}$  is required for transmitter release<sup>12</sup>, but not for carrier-mediated efflux<sup>13</sup>, this finding agrees with previous evidence<sup>8</sup> that the glutamate released from fascia dentata by depolarising stimuli originates mainly from presynaptic transmitter stores.

The question remains as to whether the glutamate presumably released by stimulation of the perforant path fibres produces the excitatory postsynaptic potential (e.p.p.) recorded in the granule cell. Proof of this hypothesis requires the demonstration that glutamate and the perforant path transmitter exert physiologically and pharmacologically identical actions on the granule cell. As a first approach to this problem, we have examined the effect of the glutamate analogue, DL-2-amino-4-phosphonobutyric acid (APB), on perforant path responses in the hippocampal slice preparation<sup>6,14</sup>. APB competitively

**Table 1** Effect of APB on averaged responses to perforant path stimulation

	Amplitude of extracellular negativity (% of control)				Recovery			
	1	3	5	10 min	1	3	5	10 min
Drug (7)	61 ± 5	51 ± 3	48 ± 4	42 ± 4	56 ± 6	68 ± 6	68 ± 8	74 ± 9
No drug (5)	97 ± 2	94 ± 3	89 ± 4	82 ± 6	82 ± 7	77 ± 7	79 ± 9	76 ± 11

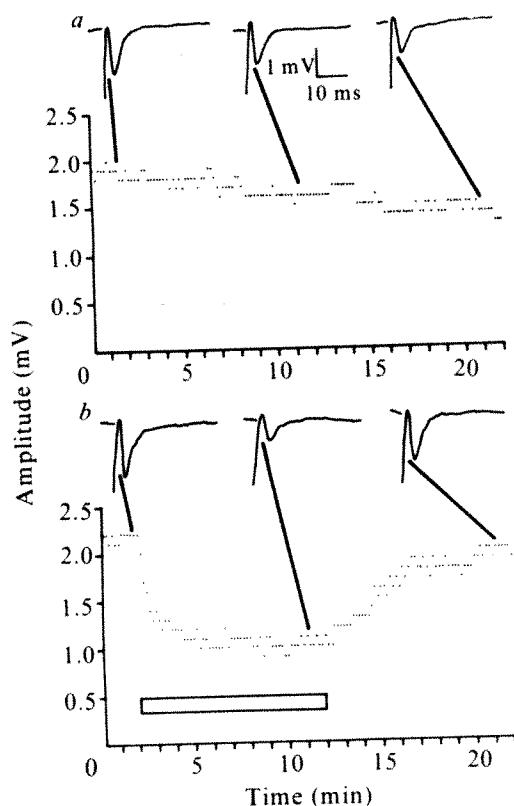
Responses of granule cell populations to perforant path stimulation were recorded as shown in Fig. 2. Amplitudes of these extracellularly recorded potentials were averaged over a period of 50 s at the times indicated. Values are expressed as mean percentages of the averaged potentials recorded within the 50-s period immediately preceding the introduction of APB into the medium ( $\pm$ s.e.m. for the number of slices in parentheses). In experiments without APB response amplitudes were averaged at comparable times after the beginning of stimulation.



and reversibly antagonises the action of glutamate iontophoretically applied to invertebrate muscle fibres<sup>15</sup> and in millimolar concentrations inhibits neuromuscular transmission<sup>16</sup>. Since invertebrate motoneurons almost certainly use glutamate as their transmitter<sup>17</sup>, APB seems to inhibit synaptic transmission mediated by glutamate. Accordingly, we stimulated the perforant path fibres in the presence and absence of 2.5 mM APB and recorded the extracellular field potentials in the molecular layer of the fascia dentata.

The results of one such experiment are shown in Fig. 2. When the perforant path fibres were continuously stimulated at 0.2 Hz, a sizeable negative potential was recorded whose amplitude declined to some extent over a 22-min period (Fig. 2a), probably as a result of habituation<sup>2,3</sup>. Introduction of APB into the superfusion medium substantially reduced the amplitude of the potential within 1 min (Fig. 2b). The amplitude recovered just as rapidly upon return to normal medium and finally reached the habituated level.

Table 1 summarises the variation of averaged extracellularly recorded potential amplitudes with time in the presence or absence of APB. By comparison with responses in normal medium averaged at similar times after the onset of stimulation, APB was seen to have been maximally effective within 3 min (46% reduction), and 3 min of washout was sufficient to terminate



**Fig. 2** Reduction of perforant path responses by APB. Slices of 0.7-mm thickness were cut transverse to the long axis of dissected hippocampi, and each was placed on a fritted polypropylene disk within a superfusion chamber. Electrodes were lowered into the chamber, and then the slice was superfused at a rate of 2.5 ml min<sup>-1</sup> with Elliott's medium<sup>19</sup> warmed to 32 °C. When the slices showed synaptic activity (1–2 h later), a bipolar stimulating electrode was inserted into the perforant path where it passes through the subiculum and a micropipette recording electrode (3–12 MΩ) into the perforant path terminal zone. The perforant path fibres were stimulated continuously with square wave pulses of 0.05 ms duration at 0.2 Hz and an intensity just below that which evoked a population spike. *a*, Amplitude of every second extracellularly recorded synaptic potential (dots) in a single experiment without drug; *b*, results of an experiment in which 2.5 mM APB was present in the medium during the period denoted by the open bar. Examples of recorded potentials are shown at the times they were evoked.

the effect of the drug. This result indicates, as expected, a readily reversible antagonism.

To determine whether APB exerted merely a local anaesthetic action, we determined its effect on the antidromic potential elicited by stimulation of the mossy fibres (granule cell axons). In four experiments APB did not alter the amplitude or form of the extracellularly recorded potentials, thus ruling out the possibility that APB acted by reducing the excitability of the granule cells. Also in several experiments APB was seen not to affect presynaptic fibre potentials.

APB probably did not act on the presynaptic boutons, since at 2.5 mM it did not reduce the quantity of glutamate released by elevated K<sup>+</sup> or affect the high affinity transport of glutamate. It, therefore, seems to exert its effect at the postsynaptic level, suggesting a pharmacological identity between glutamate and the perforant path transmitter. This conclusion must be tentative, however, until it can be rigorously shown that APB acts as a glutamate antagonist at this particular synapse.

The results of this and previous studies suggest that perforant path boutons release glutamate from a transmitter store and that the glutamate so released might then depolarise the granule cell. Thus, glutamate is most likely the transmitter used at the perforant path-granule cell synapse. Our results with APB, moreover, suggest that this antagonist will prove a useful tool in dissecting the mechanisms which underlie synaptic plasticity at this site.

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1. Mays, L. E. & Best, P. J. *Expl Neurol.* **47**, 268–279 (1975).
2. Alger, B. E. & Teyler, T. J. *Brain Res.* **110**, 463–480 (1976).
3. Teyler, T. J. & Alger, B. E. *Brain Res.* **115**, 413–425 (1976).
4. Bliss, T. V. P. & Gardner-Medwin, A. R. *J. Physiol., Lond.* **232**, 357–374 (1973).
5. Bliss, T. V. P. & Lomo, T. J. *J. Physiol., Lond.* **232**, 331–356 (1973).
6. Douglas, R. M. & Goddard, G. V. *Brain Res.* **86**, 205–215 (1975).
7. Nadler, J. V., White, W. F., Vaca, K. W., Redburn, D. A. & Cotman, C. W. *J. Neurochem.* **29**, 279–290 (1977).
8. Sandoval, M. E., Horch, P. & Cotman, C. W. *Brain Res.* (in the press).
9. Nadler, J. V., Vaca, K. W., White, W. F., Lynch, G. S. & Cotman, C. W. *Nature* **260**, 538–540 (1976).
10. Nadler, J. V., White, W. F., Vaca, K. W. & Cotman, C. W. *Brain Res.* **131**, 141–158 (1977).
11. Storm-Mathisen, J. *Brain Res.* **120**, 379–386 (1977).
12. Rubin, R. P. *Calcium and the Secretory Process* (Plenum, New York, 1974).
13. Bennett, J. P., Logan, W. J. & Snyder, S. H. *J. Neurochem.* **21**, 1533–1550 (1973).
14. Richards, C. D. & White, A. E. *J. Physiol., Lond.* **252**, 241–257 (1975).
15. Cull-Candy, S. G., Donnellan, J. F., James, R. W. & Lunt, G. G. *Nature* **262**, 408–409 (1976).
16. Clements, A. N. & May, T. E. *J. exp. Biol.* **61**, 421–442 (1974).
17. Gerschenfeld, H. M. *Physiol. Rev.* **53**, 1–119 (1973).
18. Graham, L. T. & Aprison, M. H. *Analyt. Biochem.* **15**, 487–497 (1966).
19. Elliott, K. A. C. in *Handbook of Neurochemistry* (ed. Lajtha, A.) **2**, 103–114 (1969).

## Regulation of prolactin release by endogenous opiates

ENDORPHINS, the endogenous peptides recently isolated and identified in brain have been implicated in regulation of pain<sup>1–4</sup>. But their wide distribution throughout the brain<sup>5–7</sup> and their profound behavioural effects after central administration<sup>8</sup> suggest an involvement in other central nervous system processes. Immunohistochemical identification of



these endorphins (refs 9, 10 and F. Bloom, personal communication) in hypothalamic neurones indicated that neuroendocrine effects are probable. Morphine was previously reported to block ovulation, while more recently, morphine<sup>11</sup> and endorphins<sup>12-14</sup> were observed to stimulate release of prolactin and growth hormone. Yet, these observations have not established a direct, tonic participation of endorphins in hypothalamic and anterior pituitary function. The absence of any direct action of opiate antagonists has been taken as an argument against any tonic role of endorphins. Recent reports indicate, however, that opiate antagonists do modify on-going central processes. For example, naloxone and naltrexone lower pain threshold in appropriate conditions in man and experimental animals<sup>15-18</sup>. Using naltrexone as a tool to block opiate receptor function, we have explored whether endorphins are tonically involved as a putative neurotransmitter in the regulation of prolactin release. The results presented here demonstrate a new instance where an opiate antagonist modifies normal function. The data agree with a preliminary report indicating that naloxone reduced prolactin release in immature female rats<sup>19</sup>.

As shown in Table 1, naltrexone at a dose of 1 mg per kg body weight produced a 50% decrease of serum prolactin concentration of male rats in 20 min. This decrease lasted for more than 2 h and was observed when blood was collected by decapitation or by cardiac puncture. The prolactin decrease elicited by naltrexone was the same in rats with a deafferented hypothalamus (Table 1), so we infer that the inhibition of prolactin release reflects a direct effect of naltrexone at the hypothalamic-pituitary level. Large amounts of  $\beta$ -endorphin are reported to be present in the pituitary<sup>20-22</sup>. We have examined whether naltrexone inhibits prolactin release from isolated pituitary gland *in vitro*. When pituitary halves were incubated without or

with naltrexone in concentrations up to  $10^{-4}$  M, the prolactin release was similar in the treated and untreated halves. The ability of naltrexone to reduce prolactin release stimulated by various mechanisms was also studied. The models used were: (1) chronically elevated prolactin release in oestrogen-primed ovariectomised rats; (2) prolactin release elicited by foot shock in male rats; (3) prolactin release elicited by intrahypothalamic injections of  $\beta$ -endorphin in intact rats or by systemic injection of morphine in deafferented rats; and (4), prolactin release elicited by a dopamine receptor blocker or monoamine depletor.

As shown in Table 1, naltrexone had different effects in these four models. In oestrogen-primed ovariectomised rats, naltrexone at 0.25 mg per kg intraperitoneally (i.p.) produced a significant (48%) inhibition of serum prolactin concentration. Doses of naltrexone of 1 or 5 mg per kg i.p. produced approximately a 60% decrease of hormone secretion. In addition, naltrexone is a very powerful inhibitor of the prolactin release occurring after foot shock (Table 1). It has been reported that intraventricular or systemic injection of  $\beta$ -endorphin or morphine stimulates the release of prolactin in rat<sup>12,23</sup>. To demonstrate that naltrexone acts in the hypothalamus to interfere with the opiate receptor agonist, we injected  $\beta$ -endorphin into the mediobasal hypothalamus (cannula placement was verified histologically) and naltrexone i.p. The release of prolactin induced by 1  $\mu$ g of  $\beta$ -endorphin can be blocked by 1 mg naltrexone per kg, i.p., injected 10 min previously (Fig. 1a). Similarly, the increase of serum prolactin induced by morphine in animals with a deafferented hypothalamus was blocked by naltrexone (Fig. 1b). In contrast, naltrexone failed to inhibit the prolactin release (see Table 1) elicited by haloperidol, a powerful inhibitor of dopamine receptors or by reserpine, a depletor of central monoamine stores.

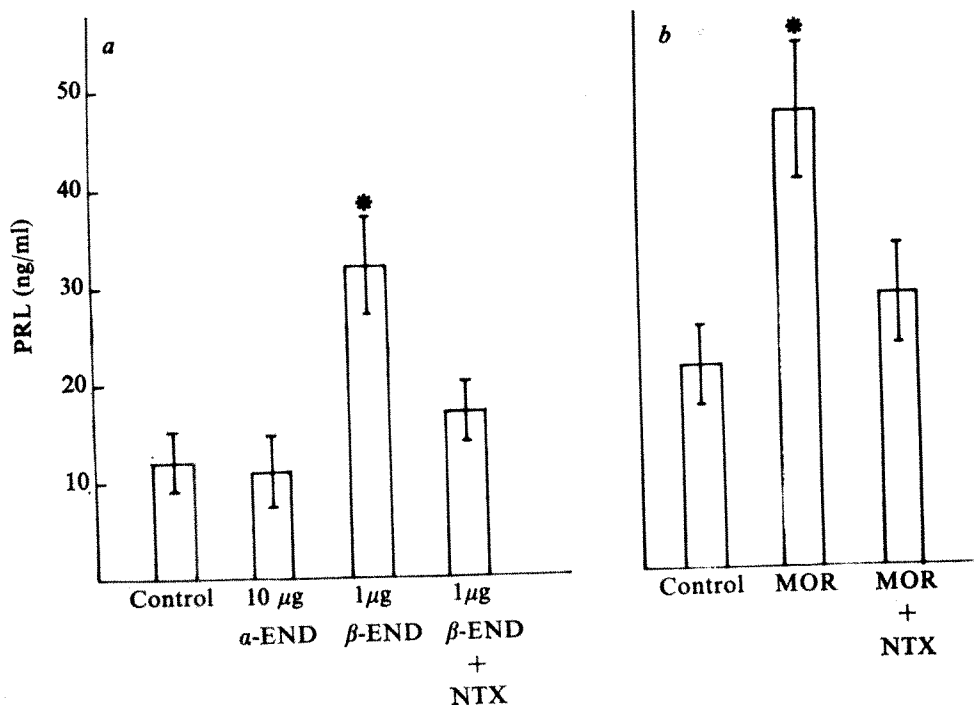
These observations show that  $\beta$ -endorphin stimulates

Table 1 Effects of naltrexone on prolactin (PRL) release

Model	Sex	Method of blood sampling	Naltrexone (mg per kg)	PRL (ng ml <sup>-1</sup> )		% Change
				Before treatment	After treatment	
Intact (n = 8)	M	Decapitation	0		11 ± 3.2	
			1		3.6 ± 1.5*	32
Intact (n = 9)	M	Ether-cardiac puncture	0	28 ± 5.4	35 ± 12.3	125
			0.25	36 ± 5.2	26 ± 5.3	87
			1.0	27 ± 3.7	18 ± 4.4*	54
			5.0	35 ± 5.3	17 ± 3.8*	49
Hypothalamic deafferentation (n = 6)	M	Ether-cardiac puncture	0	22 ± 4.7	19 ± 5	91
			1	20 ± 3.1	8.7 ± 3*	42
Oestrogen primed ovariectomised (n = 6)	F	Ether-cardiac puncture	0	180 ± 66	230 ± 40	126
			0.25	180 ± 49	85 ± 53*	52
			1.0	240 ± 44	130 ± 47*	43
			5.0	220 ± 58	90 ± 21*	33
Foot shock (n = 9)	M	Decapitation	0		73 ± 5.4	
			1		31 ± 5.7*	42
Haloperidol (0.25 mg per kg i.p.) (n = 7)	M	Decapitation	0		68 ± 7.9	
			1		55 ± 4.3	81
Reserpine 2 mg per kg i.p. (n = 9)	M	Decapitation	0		44 ± 3	
			1		45 ± 6	102

Intact, Sprague-Dawley, male rats (180-250 g body weight), deafferented male rats 6-15 d after hypothalamic isolation<sup>24</sup> or ovariectomised female rats (30 d after surgery) primed with 5  $\mu$ g oestradiol benzoate for 5 d were used. Male rats given haloperidol (0.25 mg per kg, i.p.) or reserpine (2 mg per kg, i.p.) were used 45 min and 6 h later. Foot shock (1 mA, 1-s shock per 5 s) was administered for 10 min immediately before killing. Blood samples were collected by decapitation 20 min after i.p. naltrexone or 0.85% NaCl injection or in other cases, by cardiac puncture under light ether anaesthesia before and 20 min after naltrexone or vehicle injection. Serum was separated after overnight clotting at 4 °C. Prolactin was measured using the NIAMD rat prolactin radioimmunoassay kit provided by NIAMD-NIH Pituitary Hormone Program. Mean serum prolactin (PRL) concentration of naltrexone treated rats was compared to control rats and considered significantly different if  $P < 0.05$ . Treatment effects are described as  $\bar{x}$  serum prolactin concentration of naltrexone treated rats/ $\bar{x}$  serum prolactin concentration of controls  $\times 100$  when rats were decapitated. In experiments where pre- and post-treatment samples were collected, a post-treatment/pre-treatment % was calculated for each rat and mean percentages (post/pre-treatment  $\times 100$ ) were then calculated for each group. In this group statistical significance was evaluated according to the Student's paired *t* test. *n*, No. of animals per group. \* $P < 0.05$ .

**Fig. 1 a**, Male rats (150–180 g) were implanted with a guide cannula in the mediobasal hypothalamus (4 mm A–P, 0.5 L, 8.6 D–V; König and Klippel<sup>26</sup>) and injected 3–5 d later with endorphin or vehicle in a volume of 1  $\mu$ l in 2 min. Naltrexone (1 mg per kg, i.p.) or 0.85% NaCl was given 10 min before endorphin injection. Blood was collected by decapitation. Cannula position was verified histologically after decapitation. **b**, Male rats (250–300 g) were used 8–16 d after hypothalamic isolation<sup>25</sup>. Rats were given, systemically, naltrexone in 0.85% NaCl and morphine sulphate or 0.85% NaCl at 10 min and blood was collected by cardiac puncture under light ether anaesthesia. Completeness of deafferentation was determined from histological sections of the brain. PRL, prolactin;  $\alpha$ -END,  $\alpha$ -endorphin;  $\beta$ -END,  $\beta$ -endorphin; NTX, naltrexone; MOR, morphine.



prolactin release by activation of opiate receptors. More importantly, these experiments indicate that the release of prolactin in physiological conditions can be reduced by a blockade of opiate receptors and suggest that endogenous opiate receptor ligands participate tonically in the regulation of anterior pituitary hormone secretion during basal conditions or when release is stimulated by oestrogen or by excitation of the hypothalamus as during stress.

In agreement with histoimmunological localisation of endorphins (refs 9, 10, and F. Bloom, personal communication) the action of these peptides can be located in the mediobasal hypothalamus. Naltrexone fails to inhibit the prolactin release from the pituitary but it is effective when the mediobasal hypothalamus is attached to the pituitary. It seems that the arcuate nucleus median eminence dopaminergic tract may be involved in the action of opiates since its inactivation pharmacologically prevents the action of naltrexone on prolactin release. Indeed, Ferland *et al.*<sup>24</sup> have shown that enkephalin may affect these dopaminergic neurones. It is possible that endorphins and dopamine regulate prolactin release according to an arrangement in which the two neurones act in series. Although further studies are needed to establish the precise role of hypothalamic endorphins in the regulation of prolactin release, these experiments provide strong evidence suggesting their participation.

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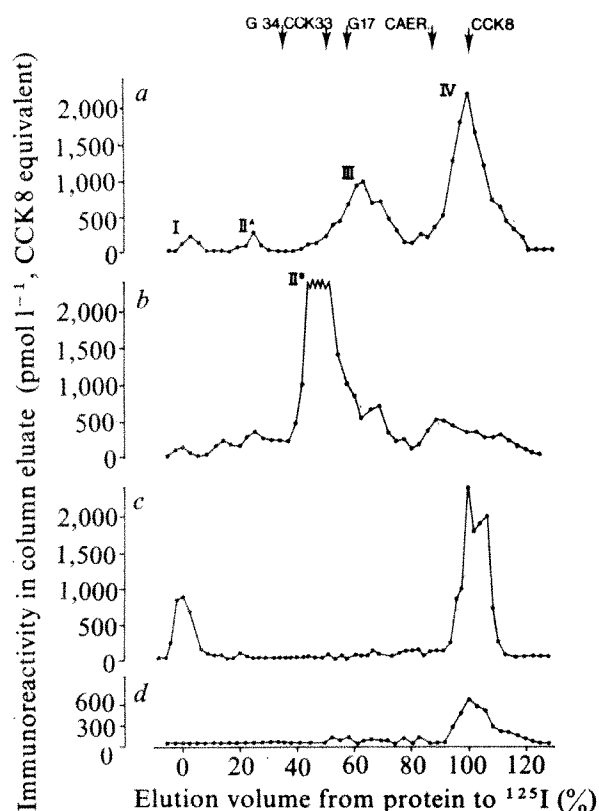
- Loh, H. H., Tseng, L. F., Wei, E. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2895–2898 (1976).
- Hughes, J. *et al.* *Nature* **258**, 577–579 (1975).
- Ling, N. & Guillemin, R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3308–3310 (1976).
- Belluzzi, J. D. *et al.* *Nature* **260**, 265–266 (1976).
- Simantov, R. & Snyder, S. H. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H.) 41–48 (Elsevier, Amsterdam, 1976).
- Wahlstrom, A., Johansson, L. & Terenius, L. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H.) 49–56 (Elsevier, Amsterdam, 1976).
- Yang, H.-Y., Hong, J. S. & Costa, E. *Neuropharmacology* **16**, 303–307 (1977).
- Bloom, F., Segal, D., Ling, N. & Guillemin, R. *Science* **194**, 630–632 (1976).
- Elde, R., Hokfelt, T., Johansson, O. & Terenius, L. *Neuroscience* **1**, 349–353 (1976).
- Simantov, R., Kuhar, M. J., Uhl, G. R. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2167–2171 (1977).

- Ojeda, S. R., Harms, P. G. & McCann, S. M. *Endocrinology* **95**, 1694–1703 (1974).
- Rivier, C., Vale, W., Ling, N., Brown, M. & Guillemin, R. *Endocrinology* **100**, 238–241 (1977).
- Lien, E. L., Fenichel, R. L., Garsky, U., Sarantakis, D. & Grant, N. H. *Life Sci.* **19**, 837–840 (1976).
- Dupont, A., Cusan, L., Garon, M., Labrie, F. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 358–359 (1977).
- Akil, H., Mayer, D. J. & Liebeskind, J. C. *Science* **191**, 961–962 (1976).
- Mayer, D. J. *Neurosci. Res. Program Bull.* **13**, 94 (1975).
- Jacobs, J. J., Tremblay, E. C. & Colombel, M. C. *Psychopharmacology*, **37**, 217–223 (1974).
- Madden, J., Akil, H., Patrick, R. L. & Barchas, J. D. *Nature* **265**, 358–360 (1977).
- Shaur, C. J., Frederickson, R. C. A., Dininger, N. B., Clemens, J. A. & Hull, R. H. *Fedn Proc.* **36**, 311 (1977).
- Lazarus, L. H., Ling, W. & Guillemin, R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2156–2159 (1976).
- Bradburg, A. F., Smyth, D. G., Shepp, C. R., Birdsall, N. J. M. & Hulme, E. C. *Nature* **266**, 793–794 (1976).
- Li, C. H. & Chung, D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1145–1148 (1976).
- Dupont, A. *et al.* *Fedn Proc.* **36**, 311 (1977).
- Ferland, L., Fuxe, K., Eneroth, P., Gustafsson, J.-A. & Skett, P. *Eur. J. Pharmacol.* **43**, 89–90 (1977).
- Halasz, B. & Pupp, L. *Endocrinology* **77**, 553–562 (1965).
- König, J. F. R. & Klippel, R. A. *The Rat Brain* (Krieger, New York, 1967).

## Immunoreactive component resembling cholecystokinin octapeptide in intestine

MANY hormones exist in several molecular forms. The intestinal hormone cholecystokinin has been isolated from hog duodenum in the form of peptides of 33 and 39 amino acid residues (CCK33 and CCK39)<sup>1–4</sup>. Extracts of hog cerebral cortex have been shown to contain components with CCK-like immunoreactivity<sup>5</sup>, but on gel filtration the main component in brain had the properties of the COOH-terminal octapeptide (CCK8) of CCK33 and CCK39<sup>6</sup>. Several other hormonal and related peptides probably occur in identical forms in brain and gut<sup>6</sup>, so it seemed possible that a CCK8-like factor would also be found in intestine. Synthetic CCK8 is known to be about twice as potent on a molar basis as CCK33 on the main target organs of the hormone (gall bladder and exocrine pancreas)<sup>7</sup> so that the possible existence of CCK8 in the intestine is of physiological significance. We have studied immunoreactive CCK-like factors in extracts of hog duodenum and jejunum and we report here the presence of relatively high concentrations of a CCK8-like component. This factor was poorly extracted by the method used to purify CCK33 and CCK39 which may explain why it was previously overlooked.

Mutt and Jorpes obtained CCK33 and CCK39 from hog intestine by first immersing the tissues in boiling water to inactivate proteases, and then transferring them to acetic acid for extraction<sup>1,2</sup>. Basic peptides in the extract, including CCK33 and CCK39, were adsorbed on alginic acid and eluted with HCl. In the present study pieces of hog duodenum, jejunum, antral mucosa and cerebral cortex were extracted either in boiling water or in boiling 0.5 M acetic acid. The tissues (0.5 g ml<sup>-1</sup>) were briefly boiled (2–3 min), homogenised and centrifuged (2,000g, 10 min). When acid extracts of duodenum or jejunum were fractionated on Sephadex G-50 the main peak of immunoreactivity in the column eluates detected by radioimmunoassay using an antiserum which cross-reacts with CCK8 and CCK33, emerged in the position of CCK33 (Fig. 1, component II<sup>B</sup>). This component could be adsorbed on alginic acid and eluted with HCl and is therefore compatible with CCK33 obtained by Mutt and Jorpes. On gel filtration, boiling water extracts of intestine contained little or no activity resembling CCK33 but did contain two other major peaks of immunoreactivity (Fig. 1, components III and IV), which would presumably be discarded in the first step of the Mutt and Jorpes method. The larger of the two peaks (IV) eluted in the region of CCK8 and the other emerged between calibration standards of CCK8 and CCK33 and is therefore probably of intermediate size. Both factors could be ad-



**Fig. 1** Elution profiles of boiling water (neutral) and boiling 0.5 M acetic acid extracts of hog jejunum and cerebral cortex on Sephadex G-50 (1 × 100 cm). In each case the equivalent of 0.5 g tissue was applied to the column. Immunoreactivity in the column eluates was estimated by radioimmunoassay using an antiserum (C) raised against CCK8 and <sup>125</sup>I-labelled CCK8; with this antiserum the ratio of immunoreactivity CCK8:CCK33, was 1.0:0.60 on a molar basis; immunoreactivity in column eluates is expressed in terms of standard CCK8. Elution volumes are expressed as % from protein peak (0%) estimated by absorption of 280 nm, to <sup>125</sup>I (100%) added as a marker to all samples. In separate runs the columns were calibrated with pure natural human big gastrin (G34), little gastrin (G17), pure natural porcine CCK33 and CCK39, synthetic caerulein (Caer) and CCK8. Elution volumes of these standards are indicated by arrows; CCK 39 emerges in the same position as CCK 33. a, Intestine neutral extract; b, intestine acid extract; c, brain neutral extract; d, brain acid extract.

**Table 1** Concentration (pmol ml<sup>-1</sup>) of intestinal peaks III and IV in samples pooled from Sephadex G-50 eluates and estimated by four COOH-terminal specific antisera and pure human G17 or CCK8 standards

	Standard	Antiserum			
		C	2716	1296	L2
Component III	G17	60.00	7.60	2.90	0.70
	CCK8	18.30	47.00	30.00	63.00
Component IV	G17	9.80	0.80	0.40	0.05
	CCK8	3.20	2.60	3.20	4.40

Antiserum C was used with <sup>125</sup>I-labelled CCK8 when CCK8 was the standard, and with <sup>125</sup>I-labelled G17 when G17 was the standard. The other antisera were raised against G17 and used with <sup>125</sup>I-labelled G17 for both G17 and CCK8 standards. Dilution curves of the brain extracts were parallel to the standard curve in each case.

sorbed on DEAE and eluted with ammonium carbonate suggesting that they are acidic peptides. Minor components (I and II<sup>A</sup>) were present in both acid and boiling water extracts (Fig. 1). Intestinal components I and IV emerged in the same position as factors previously identified in brain extracts<sup>3</sup>. Immunoreactivity corresponding to intestinal components II and III was present in negligible amounts in brain, however (Fig. 1). The failure to find significant quantities of a CCK33-like component in brain is not likely to be due to degradation during extraction for standard CCK33 was recovered in good yield (> 80%) when added to acid extracts of brain. Similarly the different patterns of components in acid and boiling water extracts of intestine are not likely to arise from interconversion during extraction, because when the residue of boiling water extracts was re-extracted with 0.5 M acetic acid CCK33-like activity was obtained in good yield (> 80%) compared to direct acid extraction.

Gastrin and CCK share the same COOH-terminal pentapeptide and antisera specific for the COOH-terminus of one hormone frequently cross-react with the other. But, immunochemical studies with antisera of different specificity support the suggestion that intestinal components III and IV are COOH-terminal fragments of CCK. Gastrin antisera (1295 and L6) which do not cross-react with CCK<sup>8,9</sup>, revealed negligible amounts of immunoreactive material in extracts of duodenum, jejunum or brain (< 0.5 pmol g<sup>-1</sup>), but high concentrations in antral mucosa (> 2 nmol g<sup>-1</sup>). Furthermore, in radioimmunoassays using four COOH-terminal specific antisera which cross-react to different degrees with heptadecapeptide gastrin (G17) and CCK8, there were only minor differences between antisera (two- to fourfold) in estimating concentrations of components III and IV when referred to a standard of CCK8 (Table 1). In contrast, when G17 was used as a standard there were up to 200-fold differences in estimates made with different antisera. Similar results have been obtained using a variety of COOH-terminal fragments of gastrin and CCK as standards; together the data indicate that with this panel of antisera the pattern of cross-reactivity of the intestinal components is readily distinguishable from gastrin-like but not CCK-like peptides.

Intestinal components II and III could also be distinguished from G17 and smaller gastrin-like peptides because they were converted by trypsin to a component emerging just before CCK8 (90% elution volume) on Sephadex G-50 whereas G17 and small fragments are resistant to trypsin. A similar tryptic peptide was obtained by trypsinisation of pure CCK33, and probably corresponds to the COOH-terminal dodecapeptide previously identified as a product of mild trypsinisation of CCK33<sup>3,3</sup>. Intestinal component IV, like CCK8, was resistant to trypsin.

Estimation of the absolute concentration of the different intestinal components must await their purification and

characterisation. But, in radioimmunoassays using antisera raised against CCK8, and the appropriate standard (CCK8 or CCK33), it was estimated from immunoreactivity in column eluates that concentrations of the CCK8-like factor in duodenum ( $40 \text{ pmol g}^{-1}$ ) were about twice those of CCK33 ( $19 \text{ pmol g}^{-1}$ ). In jejunum, on the other hand, concentrations of the CCK8-like factor ( $15 \text{ pmol g}^{-1}$ ) were about half those of CCK33 ( $27 \text{ pmol g}^{-1}$ ). Using CCK8 standard the concentration of component III was estimated to be  $20 \text{ pmol g}^{-1}$  in duodenum and  $10 \text{ pmol g}^{-1}$  in jejunum. Because COOH-terminal fragments of CCK larger than the heptapeptide have strong CCK-like actions<sup>7</sup>, the intestinal CCK8-like factor could make an important, possibly even the major, contribution to the biological activity of cholecystokinin released from the duodenum.

Several interesting questions are posed by the different patterns of distribution of CCK-like peptides in brain and gut. It is now recognised that many polypeptide hormones are synthesised via large precursor molecules which are converted to smaller active molecules by proteolytic cleavage<sup>10</sup>. Thus the different forms of CCK could be derived from precursor which was cleaved at different positions in brain and intestine. Even within the small bowel there may be differences in the patterns of biosynthetic processing of CCK, for there were differences in the relative amounts of CCK8- and CCK33-like activity in the duodenum and jejunum. For many hormones, for example, insulin and parathyroid hormone, the site at which the prohormone is cleaved to yield the active hormonal peptide consists of two or more consecutive basic amino acid residues, and is susceptible to digestion by trypsin-like enzymes<sup>10</sup>. Only a single basic residue (arginine) links CCK8 to the NH<sub>2</sub>-terminal portion of CCK33, however, and this position is relatively resistant to trypsin. If, as seems likely, CCK33 is a prohormone for CCK8 then conversion is probably mediated by a specific enzyme.

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- Mutt, V. *Arkiv f. Kemi*, **15**, 69-74 (1959).
- Mutt, V. & Jorpes, E. in *Secretin, Cholecystokinin, Pancreozymin and Gastrin*, 1-179 (Springer Berlin, 1973).
- Mutt, V. & Jorpes, E. *Eur. J. Biochem.*, **6**, 156-162 (1968).
- Mutt, V. *Clin. Endocrinol.*, **5**, suppl. 175s-183s (1976).
- Dockray, G. J. *Nature*, **264**, 568-570 (1976).
- Pearse, A. G. E. *Nature*, **262**, 92-94 (1976).
- Grossman, M. I. in *Peptide Hormones* (ed. Parsons, J. A.) 106-116 (Macmillan, London, 1976).
- Dockray, G. J. & Walsh, J. H. *Gastroenterology*, **68**, 222-230 (1975).
- Dockray, G. J. & Taylor, I. L. *Gastroenterology*, **71**, 971-977 (1976).
- Steiner, D. F. in *Peptide Hormones* (ed. Parsons, J. A.) 49-64 (Macmillan, London, 1976).

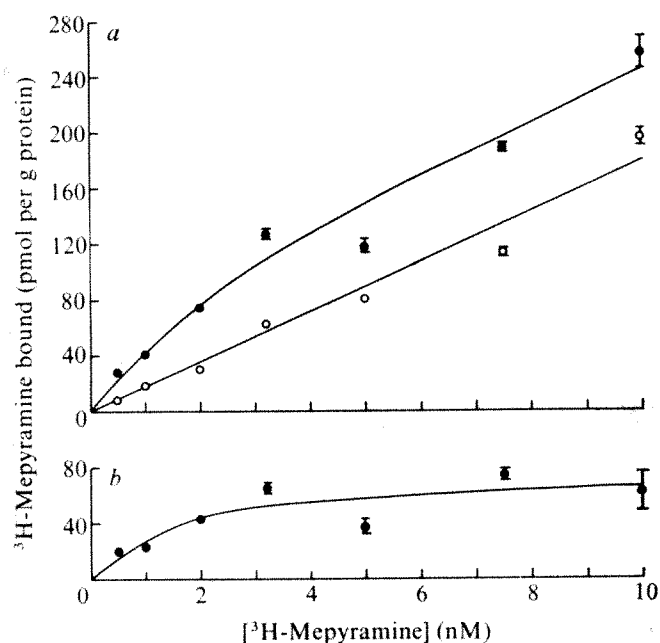
## Specific binding of <sup>3</sup>H-mepyramine to histamine H<sub>1</sub> receptors in intestinal smooth muscle

THE specific binding of high-affinity ligands to a wide range of pharmacological receptors has been used to obtain information on receptor numbers, distribution and the characteristics of agonist and antagonist binding. The first study of this type, by Paton and Rang<sup>1</sup>, was of the binding of <sup>3</sup>H-atropine to muscarinic receptors in guinea pig intestinal smooth muscle. The histamine H<sub>1</sub> receptor has not previously been studied,

and here we describe the specific binding of mepyramine, an H<sub>1</sub>-selective antagonist<sup>2</sup>, to histamine H<sub>1</sub> receptors in homogenates of the longitudinal muscle-myenteric plexus preparation from guinea pig small intestine.

<sup>3</sup>H-Mepyramine was synthesised by catalytic hydrogenation with tritium gas of the 5-bromopyridyl derivative. The method will be described in detail elsewhere. The purity of the product was established by the identity with authentic non-radioactive mepyramine of (1) the *R<sub>F</sub>* value on thin-layer chromatography in three solvent systems, (2) the mobilities on high-voltage electrophoresis and (3) the ultraviolet spectrum. The affinity constant of <sup>3</sup>H-mepyramine for the histamine H<sub>1</sub> receptor,  $1.6 \pm 0.4 \times 10^9 \text{ M}^{-1}$  (three measurements), deduced from the parallel shift of the log dose-response (contraction) curve of longitudinal muscle strips to histamine, was not significantly different from that of non-radioactive mepyramine,  $1.2 \pm 0.3 \times 10^9 \text{ M}^{-1}$  (six measurements), the figures being in accord with values in the literature<sup>2-4</sup>. The concentration of <sup>3</sup>H-mepyramine was determined from the absorbance at 310 nm. The specific activity of the purified product was  $20 \text{ Ci mmol}^{-1}$ .

The binding of <sup>3</sup>H-mepyramine, either alone or in the presence of  $2 \times 10^{-6} \text{ M}$  non-radioactive mepyramine, to homogenates of longitudinal muscle strips from guinea pig small intestine and the difference curve, which is taken to represent the receptor-specific binding, is shown for a representative experiment in Fig. 1. The difference between all pairs of points was statistically significant ( $P < 0.05$ ). The best-fit<sup>5</sup> parameters for the experiment in Fig. 1, assuming binding follows simple mass-action kinetics, were  $1.7 \pm 1.0 \text{ nM}$  for *K<sub>d</sub>*, the dissociation



**Fig. 1** *a*, Binding of <sup>3</sup>H-mepyramine to homogenates of guinea pig intestinal smooth muscle. Longitudinal muscle strips from guinea pig small intestine, prepared essentially as described by Rang<sup>10</sup>, were chopped with scissors, partially homogenised in five volumes of 50 mM sodium-potassium phosphate buffer, pH 7.5, in a glass homogeniser with a glass pestle and the ice-cold suspension then treated twice with a Polytron blender (setting 5 for 15 s). Homogenate (0.2 ml, approximately 2 mg protein) in 1.8 ml 50 mM sodium-potassium phosphate buffer, pH 7.5, was incubated with various concentrations of <sup>3</sup>H-mepyramine in the presence (○) or absence (●) of  $2 \times 10^{-6} \text{ M}$  mepyramine for 60 min at 30 °C. Aliquots (0.25 ml) were centrifuged at 8,700g for 60 s in a Beckman Microfuge B and the pellet washed superficially twice with 0.1 ml ice-cold phosphate buffer. The tritium in the pellet was determined by liquid scintillation counting. Values are means  $\pm$  s.e. of quadruplicate determination of two to four incubations. *b*, Receptor-specific binding; obtained by subtracting the binding of <sup>3</sup>H-mepyramine in the presence of  $2 \times 10^{-6} \text{ M}$  mepyramine from the uninhibited binding. The curve is a best-fit curve calculated by the method of Wilkinson<sup>5</sup>.



constant and  $77 \pm 15$  pmol per g protein for the number of binding sites. The best-fit values obtained by combining data from a number of such experiments were  $1.3 \pm 0.3$  nM and  $68 \pm 7$  pmol per g protein, although the maximum amount of  $^3\text{H}$ -mepyramine binding seemed to vary between homogenates (compare Table 2). The affinity constant,  $7.7 \pm 1.8 \times 10^8 \text{ M}^{-1}$  ( $1/K_d$ ) is in reasonable agreement with the value,  $1.6 \pm 0.4 \times 10^9 \text{ M}^{-1}$ , obtained from inhibition of the contractile response.

To establish that the binding of  $^3\text{H}$ -mepyramine sensitive to inhibition by  $2 \times 10^{-6} \text{ M}$  mepyramine does represent receptor-specific binding we have examined the inhibition of  $^3\text{H}$ -mepyramine binding by three compounds considered to be selective antagonists at histamine  $\text{H}_1$  receptors, chlorpheniramine, promethazine and triprolidine, and by two drugs having their primary effect on other receptors, burimamide (histamine  $\text{H}_2$ ) and lachesine (muscarinic). The maximal inhibition of  $^3\text{H}$ -mepyramine binding by each drug did not differ significantly from that produced, measured on the same homogenate, by  $2 \times 10^{-6} \text{ M}$  mepyramine. The curves for the inhibition by the  $\text{H}_1$  antagonists of the receptor-specific binding approximated to hyperbolae and the affinity constants (Table 1) calculated from values for 50% inhibition compared well with those in the literature obtained from inhibition of the contractile response. The two non- $\text{H}_1$  antagonists were only weak inhibitors of  $^3\text{H}$ -mepyramine binding. The affinity constant deduced for lachesine,  $1.6 \times 10^5 \text{ M}^{-1}$ , is in good agreement with the value<sup>6</sup> from antagonism of the response to histamine,  $1.0 \times 10^5 \text{ M}^{-1}$ ,

Table 1 Inhibition of  $^3\text{H}$ -mepyramine binding

Drug	Affinity constant ( $\text{M}^{-1}$ ) from inhibition of		(ref.)
	$^3\text{H}$ -mepyramine binding*	Contraction†	
Mepyramine	$1.0 \times 10^9$	$1.2 \times 10^9$	
(±) Chlorpheniramine	$7.6 \times 10^8$	$6.6 \times 10^8$	(3)
Promethazine	$4.4 \times 10^8$	$8.5 \times 10^8$	(3)
Triprolidine	$1.0 \times 10^9$	c. $10^9$	(9)
Burimamide	c. $10^4$	$3.5 \times 10^3$	(8)
Lachesine	$1.6 \times 10^5$	$1.0 \times 10^5$	(6)

\* Incubations as in Fig. 1 with  $10^{-9} \text{ M}$   $^3\text{H}$ -mepyramine. The affinity constants,  $K_a$ , of the inhibitors were calculated from the relationship  $K_a = (K_{\text{mep}} [M] + 1)/[A]$ , where  $[A]$  is the concentration of drug required for 50% inhibition of receptor-specific  $^3\text{H}$ -mepyramine binding,  $[M]$  is the concentration of  $^3\text{H}$ -mepyramine and  $K_{\text{mep}}$  its affinity.  $K_{\text{mep}}$  was taken to be  $1.2 \times 10^9 \text{ M}^{-1}$ , the mean of the various determinations made.

† From inhibition of the contractile response to histamine of intact segments or longitudinal muscle strips from guinea pig ileum. In our own measurements  $K_a$  was obtained from the relationship for competitive inhibition: dose ratio  $- 1 = [A] \times K_a$ , where the dose ratio is the ratio of the dose of agonist necessary to achieve a given response in the presence of antagonist, concentration  $[A]$ , to the dose required with no antagonist present.

a figure four orders of magnitude lower than its affinity for the muscarinic receptor<sup>7</sup>,  $1.1 \times 10^9 \text{ M}^{-1}$ . The affinity of burimamide as an inhibitor of  $^3\text{H}$ -mepyramine binding, about  $10^4 \text{ M}^{-1}$ , is an order of magnitude less than its affinity at the  $\text{H}_2$  receptor<sup>8</sup>,  $1.3 \times 10^5 \text{ M}^{-1}$ .

The number of histamine  $\text{H}_1$  receptors in homogenates of guinea pig intestinal smooth muscle, 68 pmol per g protein, is apparently much smaller than the number of muscarinic receptors reported in this tissue<sup>11-13</sup>, but the criterion per g protein clearly makes the number obtained very dependent on the degree of purification of the membrane fraction and the assay technique employed. To make a more direct comparison between the numbers of histamine  $\text{H}_1$  and muscarinic receptors, in five experiments the maximum receptor-specific binding of  $^3\text{H}$ -mepyramine was compared on the same homogenate with that of  $^3\text{H}$ -quinuclidinyl benzilate ( $^3\text{H}$ -QNB), a muscarinic ligand<sup>14</sup>. The relative amounts of the two receptors varied within a fairly narrow range (Table 2) giving a mean value of

Table 2 Comparison of the numbers of histamine  $\text{H}_1$  and muscarinic receptors in guinea pig intestinal smooth muscle

Expt	No. of receptors (pmol per g protein)		$\text{H}_1/\text{Muscarinic} (\%)$
	Histamine $\text{H}_1$	Muscarinic	
1	82*	496	17
2	41*	407*	10
3	62*	297	21
4	93	493	19
5	45	334	13
Mean	$65 \pm 10$	$405 \pm 41$	$16 \pm 2$

The no. of receptors was estimated either (\*) from the maximum specific binding obtained from binding curves in the presence and absence of  $2 \times 10^{-6} \text{ M}$  mepyramine (for  $^3\text{H}$ -mepyramine; see Fig 1) or  $2 \times 10^{-6} \text{ M}$  methylatropinium bromide (for  $^3\text{H}$ -QNB) or (values not starred) from the receptor-specific binding with  $7.5 \times 10^{-9} \text{ M}$   $^3\text{H}$ -mepyramine or  $7.7 \times 10^{-9} \text{ M}$   $^3\text{H}$ -QNB. In each experiment measurement of the numbers of the two receptors was made on the same homogenate.  $^3\text{H}$ -QNB ( $^3\text{H}$ -quinuclidinyl benzilate), specific activity  $13 \text{ Ci mmol}^{-1}$ , was obtained from the Radiochemical Centre, Amersham.

$16 \pm 2$  for the number of  $\text{H}_1$  receptors as a percentage of the number of muscarinic receptors. This figure must still, however, be viewed with some caution. The composition of the assay medium, 50 mM sodium potassium phosphate, does not correspond to the *in vivo* environment and while the number of muscarinic receptors determined by antagonist binding seems to be insensitive to the ionic composition<sup>15</sup> it remains to be established whether this also holds for mepyramine.

As far as we are aware this is the first report of binding studies on the histamine  $\text{H}_1$  receptor using a selective high-affinity antagonist. There has been a report<sup>16</sup> that by using suitable protecting agents the histamine receptor in cat intestinal muscle could be labelled with the relatively nonspecific antagonist  $^3\text{H}$ -dibenamine ( $58.5 \text{ mCi mmol}^{-1}$ ). The amount of presumed receptor material labelled seems remarkably large, however, being some 200-fold greater than that bound by  $^3\text{H}$ -mepyramine in our experiments and indeed an order of magnitude greater than the highest reported estimate of the number of muscarinic receptors in intestinal smooth muscle. Even allowing for species differences there must be some suspicion that the bulk of the dibenamine was bound to non-receptor sites.

These observations suggest that  $^3\text{H}$ -mepyramine should be a valuable tool with which to investigate the numbers of histamine receptors in various tissues in various conditions and to study the binding characteristics of  $\text{H}_1$  agonists and antagonists.

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1. Paton, W. D. M. & Rang, H. P. *Proc. R. Soc. B* **163**, 1-44 (1965).
2. Ash, A. S. F. & Schild, H. O. *Br. J. Pharmac. Chemother.* **27**, 427-439 (1966).
3. Marshall, P. B. *Br. J. Pharmac. Chemother.* **10**, 270-278 (1955).
4. Arunlakshana, O. & Schild, H. O. *Br. J. Pharmac. Chemother.* **14**, 48-58 (1959).
5. Wilkinson, G. N. *Biochem. J.* **80**, 324-332 (1961).
6. Reasbeck, P. G. & Young, J. M. *Br. J. Pharmac.* **48**, 148-155 (1973).
7. Paton, W. D. M. & Rang, H. P. *Adv. Drug Res.* **3**, 57-80 (1966).
8. Black, J. W., Duncan, A. M., Durant, C. J., Ganellin, C. R. & Parsons, E. M. *Nature* **236**, 385-390 (1972).
9. Green, A. F. *Br. J. Pharmac. Chemother.* **8**, 171-176 (1953).
10. Rang, H. P. *Br. J. Pharmac. Chemother.* **22**, 356-365 (1964).
11. Fewtrell, C. M. S. & Rang, H. P. in *Drug Receptors* (ed. Rang, H. P.) 211-224 (Macmillan, London, 1973).
12. Burgen, A. S. V., Hiley, C. R. & Young, J. M. *Br. J. Pharmac.* **50**, 145-151 (1974).

13. Yamamura, H. I. & Snyder, S. H. *Molec. Pharmac.* **10**, 861–867 (1974).
14. Yamamura, H. I. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1725–1729 (1974).
15. Birdsall, N. J. M., Burgen, A. S. V., Hulme, E. C. & Wells, J. W. *Br. J. Pharmac.* **59**, 503P (1977).
16. Takagi, K. & Uchida, M. *Jap. J. Pharmac.* **20**, 272–286 (1970).

## Presumptive control mutation for alcohol dehydrogenase in *Drosophila melanogaster*

EXAMPLES of control mutations are rare in eukaryotes. Indeed, the only well-documented examples are in *Aspergillus*<sup>1,2</sup> and a mutant affecting xanthine dehydrogenase (XDH) activity and mapping adjacent to, but separable from, *rosy*, the structural gene for XDH in *Drosophila melanogaster*<sup>3,4</sup>. Control of alcohol dehydrogenase (ADH), particularly in *Drosophila* has recently been studied. In examining ADH activity in selection lines and in samples from natural populations, a line was found to be *Adh*<sup>S</sup> and to have half the activity of a normal slow-migrating allele. To test the hypothesis that the low activity *Adh*<sup>S</sup> line contains a control mutation, we have attempted to separate the activity phenotype from the electrophoretic (structural gene) phenotype by recombination. We have succeeded in doing this, and in the process have obtained a very accurate position for *Adh* based on its recombination with closely-linked marker loci.

ADH is a small homodimer with a molecular weight of about 24,000 (R. Ambler, personal communication). The alcohol dehydrogenase (*Adh*) locus maps at position 50.1 on the second chromosome of *D. melanogaster*, and its cytogenetic location is known within two bands (35B2–3) on the polytene chromosome map of salivary glands<sup>5</sup>.

In addition, *Adh* is highly polymorphic in nature<sup>6–8</sup>. Activity variation is also known<sup>9–11</sup> and we have identified a striking variant in a population collected near Cambridge, UK. A line derived from that population is homozygous for the slow-migrating allele (*Adh*<sup>S</sup>) but has only about half the activity of a standard *Adh*<sup>S</sup> allele, as measured by the rate at which it acts on the substrate isopropanol. Taking *Adh*<sup>F</sup> as a standard with relative activity of 1.0, we found the ratio of activities of *Adh*<sup>F</sup> to *Adh*<sup>S</sup> to 'low activity *Adh*<sup>S</sup>' to be approximately 1.0 : 0.57 : 0.28 (ref. 12). Radial immunodiffusion assays<sup>12</sup> have confirmed that the decreased activity is due to the fact that the allele produces about half as many enzyme molecules as the normal *Adh*<sup>S</sup> or *Adh*<sup>F</sup> alleles. Thus, it is a good candidate for a control mutation, though, as is the case for some mutations of XDH, this variant could affect the quantity of ADH by changing the primary sequence of the protein, and only protein chemistry can unambiguously rule out this possibility.

Let us represent the low activity allele as *Adh*<sup>S-L</sup>, the normal slow-mobility allele as *Adh*<sup>S-H</sup>, and the possible fast-mobility alleles as *Adh*<sup>F-L</sup> and *Adh*<sup>F-H</sup>, respectively. A multiply-marked second chromosome stock carrying *black* (*b*, 2–48.5), *elbow* (*el*, 2–50.0), *reduced-scraggly* (*rd*<sup>S</sup>, 2–51.2), *purple* (*pr*, 54.5), and *cinnabar* (*cn*, 2–57.5) was crossed to the *Adh*<sup>S-L</sup> stock, which is marked with the third chromosome mutation *veinlet*. The *Adh* locus maps between *el* and *rd*<sup>S</sup><sup>13</sup> and the *b el rd*<sup>S</sup> *pr cn* stock is homozygous for *Adh*<sup>F-H</sup>. Heterozygous females were backcrossed to *b el rd*<sup>S</sup> *pr cn* males and the progeny were scored for two recombinant classes: *b el* + + + and + + *rd*<sup>S</sup> *pr cn*. All other progeny were pooled and counted as non-recombinants for that region.

A total of 122 *b el* + + + and 128 + + *rd*<sup>S</sup> *pr cn* recombinant progeny were recovered among 59,738 scored. This allows us to calculate very accurately the map distance between the two morphological markers flanking the *Adh* locus. This distance is estimated to be 0.42 cM, much smaller than the estimate of 1.2 cM normally quoted<sup>13</sup>. Having such close flanking markers makes the recombinational assay of potential control mutants exceptionally accurate and efficient. Indeed, independent evidence suggests that there may be only one gene between *el* and *Adh*<sup>S</sup>.

Each + + *rd*<sup>S</sup> *pr cn* recombinant male was then mated to females heterozygous for *Df*(2L)64j (ref. 9), which includes the *el* and *Adh* loci, and for the balancer chromosome *Curly of Oster*. Homozygous + + *rd*<sup>S</sup> *pr cn* recombinant chromosomes were produced by standard crosses. A total of 35 of the 70 such chromosomes from males were successfully made homozygous. These were then tested for both ADH electrophoretic mobility and for enzyme activity.

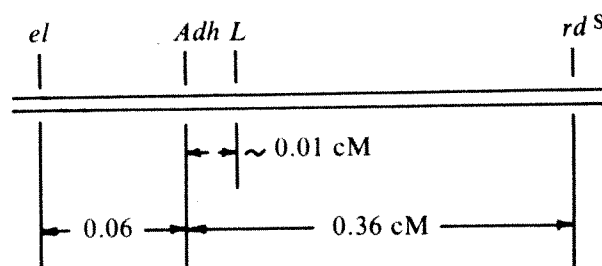


Fig. 1 Order and estimated map distances for *Adh*, the low activity site (L) and flanking markers.

A total of 30 recombinants were *Adh*<sup>S</sup>. On the basis of the earlier estimate of the map distance between *el* and *rd*<sup>S</sup>, this places the *Adh* locus very near *el*, and about 0.36 cM from *rd*<sup>S</sup> (Fig. 1). Of greater importance, however, was the observation that one of the 30 *Adh*<sup>S</sup> lines had normal enzyme activity, while the other 29 still had low activity. The complementary *Adh*<sup>F-L</sup> recombinant has not yet been recovered. Thus, an activity modifier locus has been identified that is separable from, and to the right of, the *Adh* structural gene as marked by electrophoretic mobility alleles.

Other recombinants are now being studied. But the data so far have enabled us to measure accurately the recombination distance between two loci flanking the *Adh* locus and have provided the first evidence that a *presumptive* control mutation decreasing the number of enzyme molecules is located proximal, but very close, to the structural gene for alcohol dehydrogenase.

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1. Arst, H. N. & MacDonald, D. W. *Nature* **254**, 26–31 (1975).
2. Hynes, M. J. *Nature* **253**, 210–212 (1975).
3. Chovnick, A. *et al. Genetics* **84**, 233–255 (1976).
4. Chovnick, A., Gelbart, W. & McCarron, M. *Cell* **11**, 1–10 (1977).
5. Woodruff, R. C. & Ashburner, M. (in preparation).
6. Johnson, F. M. & Burrows, P. M. *Biochem. Genet.* **14**, 47–58 (1976).
7. Vigue, C. L. & Johnson, F. M. *Biochem. Genet.* **9**, 213–227 (1973).
8. Milkman, R. *Biochem. Genet.* **14**, 383–387 (1976).
9. Grell, E. H., Jacobsen, K. B. & Murphy, J. B. *Ann. N.Y. Acad. Sci.* **151**, 441–455 (1968).
10. Sofer, W. H. & Hatkoff, M. A. *Genetics* **72**, 545–549 (1972).
11. Ward, R. D. & Hebert, P. D. N. *Nature new Biol.* **236**, 243–244 (1972).
12. Thompson, J. N., Jr & Kaiser, T. N. *Heredity* **38**, 191–195 (1977).
13. Lindsley, D. L. & Grell, E. H. *Genetic Variations of Drosophila melanogaster* (Carnegie Institution of Washington, Publ. No. 627, 1967).

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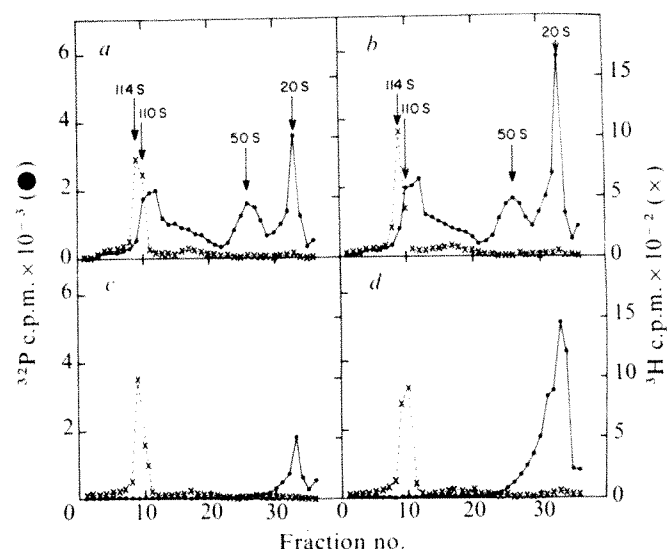
## Synthesis of infectious $\Phi$ X-174 bacteriophage *in vitro*

STUDY of virus assembly has indicated the involvement of a diverse spectrum of protein-protein and protein-nucleic acid interactions (for review see ref. 1). In *Escherichia coli* cells infected with bacteriophage  $\Phi$ X-174, synthesis of single-stranded circular viral DNA requires the functions of at least seven phage-coded genes which include genes specifying viral structural proteins F, G and H non-virion proteins A, B, C and D. The only known phage gene that is not involved in single-stranded DNA synthesis is gene E (lysis) (for review see ref. 2). It seems that synthesis of single-stranded circular viral DNA of  $\Phi$ X-174 is very tightly coupled with the phage morphogenetic pathway. The synthesis of  $\Phi$ X-174 viral DNA involves a replicative form (RF) DNA with an extended tail of single-stranded viral DNA up to one genome in length ('rolling circle' or  $\sigma$  structure DNA)<sup>3-7</sup>. Fujisawa and Hayashi<sup>8,9</sup> found that  $\sigma$  DNA in infected cells is associated with a number of phage and host proteins as a 50S complex which is a precursor of mature phage particles. They have proposed that synthesis and packaging of  $\Phi$ X-174 single-stranded DNA occur in the 50S complex. Formation of the 50S complex requires the association of a functional RF DNA template with a 108S capsomeric structure containing the protein products of genes D, F, G and H (ref. 10). The functions of genes B and C are also required for formation of the 50S complex. The gene B protein is necessary to catalyse assembly of gene F and gene G proteins<sup>11</sup> to form a precursor of the 108S structure<sup>10-12</sup>. In the absence of gene C protein, the 108S structure is produced but the 50S complex is not formed<sup>10</sup>. Apparently, RF DNA cannot act as a functional template for formation of the 50S complex and, consequently, for synthesis of single-stranded DNA if the gene C protein is absent. To elucidate the function of  $\Phi$ X-174-coded proteins during phage morphogenesis and single-stranded DNA synthesis, we have developed an *in vitro* system that synthesises circular, single-stranded viral DNA<sup>13</sup>. The system contained an extract prepared from cells infected with a lysis-defective mutant of  $\Phi$ X-174. We show here that synthesis of circular viral DNA in the system does depend on the functions of the proteins encoded by  $\Phi$ X-174 genes B and C. The circular single-stranded viral DNA synthesised *in vitro* is contained in infectious particles whose appearance is very much like that of mature  $\Phi$ X-174 phage particles when examined by electron microscopy.

To test the requirement for gene B and gene C proteins we performed an *in vitro* complementation experiment. Cell extracts were prepared from *E. coli* H570-22 (*Su<sup>-</sup>*) that had been infected with either a  $\Phi$ X-174 double mutant (*och11 am3*)<sup>14</sup> of gene C (*ochre*) and gene E (*amber*) or  $\Phi$ X-174 double mutant (*och12 am3*)<sup>14</sup> of gene C (*ochre*) and gene E (*amber*). The amber mutation in the lysis gene E was present for technical reasons and has no effect on phage DNA replication or morphogenesis. *In vitro* complementation can be performed by mixing infected cell extracts directly in the *in vitro* system but the complementary activity of such a system seems to be limited by a deficiency of phage-specific proteins. In order to increase the activity of the *in vitro* reaction, a fraction (fraction 1) enriched in  $\Phi$ X-174-specific proteins was prepared from infected cell extracts by precipitation with  $(\text{NH}_4)_2\text{SO}_4$ . *In vitro* reaction mixtures were prepared using the four possible combinations of cell extract and fraction 1, incubated with radioactive dTTP and analysed by sucrose gradient centrifugation. The two reaction mixtures containing homologous combinations of extract and fraction 1 incorporated dTTP only into material sedimenting at 20S (Fig. 1c, d). The 20S material consisted of only RF DNA<sup>13</sup>. No single-stranded DNA was synthesised in these two reaction mixtures which lack either gene B protein or gene C protein. In contrast, the two reaction mixtures containing heterologous

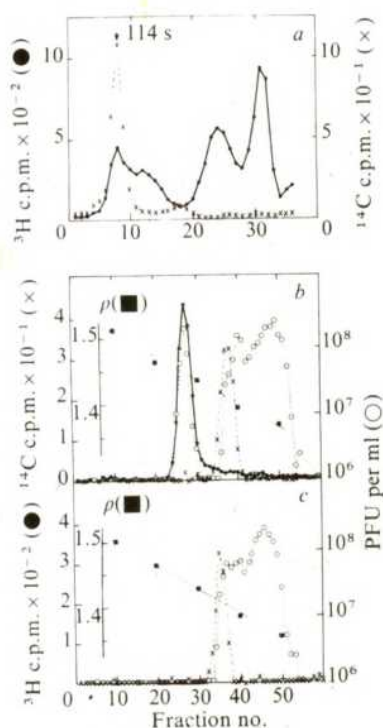
combinations of extract and fraction 1 incorporated dTTP not only in material sedimenting at 20S but also at 50S and 110S (Fig. 1a, b). Sedimentation analysis, in an alkaline sucrose gradient, of DNA isolated from the 110S material showed that the DNA consisted almost entirely of single-stranded circular DNA of unit-genome size (data not shown).

To determine infectivity associated with 110S material, DNA in the 110S material was density-labelled. A reaction mixture containing extracts and fraction 1 was prepared from both *Och11am3* infected cells and *Och12am3* infected cells and was incubated with BrdUTP substituted for dTTP. Figure 2a shows analysis by sucrose-gradient sedimentation of the products.



**Fig. 1** Sedimentation analysis of *in vitro* product by sucrose gradient centrifugation. *E. coli* H570-22 was infected with  $\Phi$ X-174 *Och11am3* or with *Och12am3*. Infected cells were collected and washed as described previously<sup>13</sup>. The washed cells were suspended in buffer A (50 mM Tris-HCl, 0.1 mM EDTA, pH 7.3, at 25 °C) containing 10% sucrose and 100 mM KCl and cell extracts were prepared. Saturated  $(\text{NH}_4)_2\text{SO}_4$  (in 50 mM Tris-HCl, 1 mM EDTA, pH 7.5) was added to the cell extract (0.8 ml) to 40% saturation, and the mixture was left at 0 °C for 30 min. The precipitate was collected by centrifugation at 18,000g for 20 min. The pellet was suspended in 0.1 ml of buffer B (20 mM Tris-HCl, 1 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 10% sucrose, 50 mM KCl, pH 7.5 at 0 °C) and dialysed for 3 h against three changes of 125 ml of buffer B. The dialysed fraction is termed fraction 1. Fraction 1 contained about 20 mg of protein per ml. A standard reaction mixture (0.25 ml) contained 0.14 ml of cell extract, 20  $\mu$ l of fraction 1, 50 mM Tris-HCl, pH 7.1 at 30 °C, 0.1 mM each dATP, dCTP, dGTP and  $\alpha^{32}\text{P}$ -dTTP (150–500 c.p.m.  $\mu\text{mol}^{-1}$ ), 0.1 mM each CTP, UTP, GTP, 0.2 mM ATP, 0.2 mM NAD, 15 mM MgCl<sub>2</sub>, 10 mM  $\beta$ -mercaptoethanol, 500  $\mu\text{g ml}^{-1}$  bovine serum albumin (BSA) and 80  $\mu\text{g ml}^{-1}$  rifampicin. Reaction mixtures were incubated at 30 °C for 18 min. A complete reaction mixture (in 62.5  $\mu$ l) ( $\alpha^{32}\text{P}$ -dTTP 220 c.p.m.  $\mu\text{mol}^{-1}$ ) containing a, 35  $\mu$ l of extract from *Och11am3* infected cells and 10  $\mu$ l of fraction 1 from *Och12am3* infected cells; b, 35  $\mu$ l of extract from *Och12am3* infected cells and 10  $\mu$ l of fraction 1 from *Och11am3* infected cells; c, 35  $\mu$ l of the extract, 10  $\mu$ l of fraction 1 both from *Och11am3* infected cells; or d, 35  $\mu$ l of the extract and 10  $\mu$ l of fraction 1 both from *Och12am3* infected cells was incubated at 30 °C for 18 min. The reaction was stopped by the addition of 150  $\mu$ l of ice-cold buffer C (50 mM Tris-HCl, 100 mM KCl, 15 mM EDTA and 500  $\mu\text{g ml}^{-1}$  BSA, pH 7.5 at 0 °C).  $^3\text{H}$ -Thymidine-labelled mature phage was added as a marker and 200  $\mu$ l of the diluted reaction mixture was layered onto a sucrose gradient (15–30% in 10 mM Tris-HCl, pH 7.25 at 25 °C, 100 mM NaCl, 5 mM EDTA and 3.5% CsCl with a 0.15 ml cushion of 55% CsCl and 50% sucrose at the bottom of the gradient). Sucrose gradient was centrifuged in Beckman SW50.1 rotor at 49,000 r.p.m. for 70 min at 0 °C. Fractions were collected onto squares of Whatman 3 MM filter paper which were washed with trichloroacetic acid (TCA) and ethanol, dried and counted. Fractions 37 to 40 were discarded because these fractions did not contain TCA-insoluble material. ●,  $^{32}\text{P}$  c.p.m.; ×,  $^3\text{H}$  c.p.m.





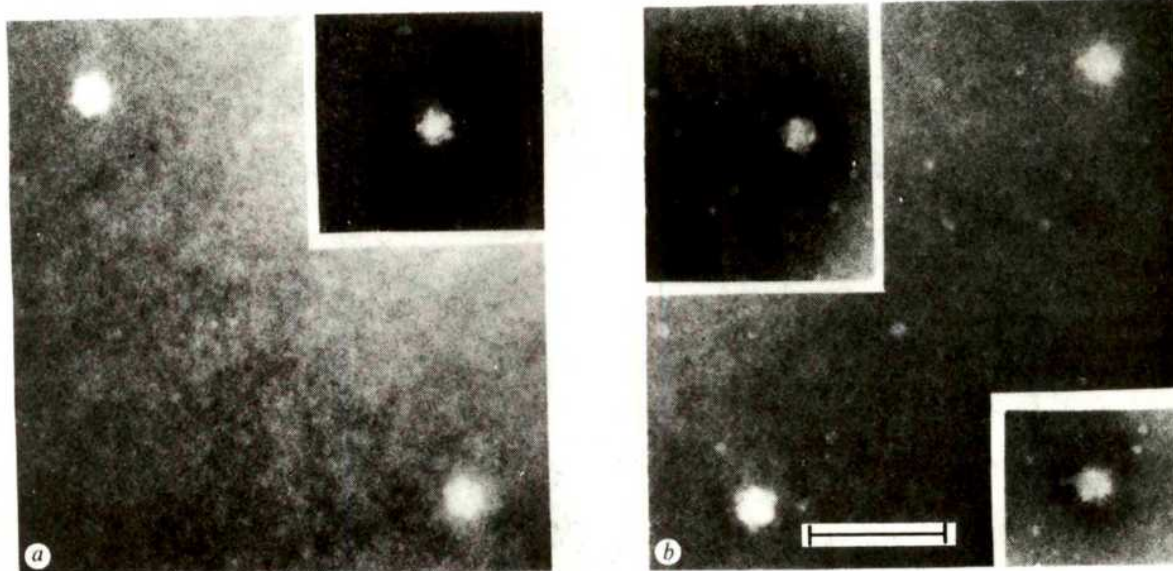
The fractions containing density-labelled 110S material (fractions 5 to 8) from the sucrose gradient shown in Fig. 2a were combined and subjected to buoyant density centrifugation in CsCl. The 110S material banded in a single peak at a density of  $1.45 \text{ g ml}^{-1}$  while mature phage particles not containing BrdUMP banded at a density of  $1.41 \text{ g ml}^{-1}$  (Fig. 2b). When each fraction of the CsCl gradient was titred using *E. coli* Su20c (*Su*<sup>+</sup>) as the indicator, a peak of infectivity which coincided exactly with the peak of radioactivity at the density of  $1.45 \text{ g ml}^{-1}$  was found. This peak of infectious material synthesised *in vitro* contained particles that were indistinguishable from mature phage particles when examined in an electron microscope (Fig. 3).

Infectious but non-radioactive material was found at densities equal to and less than the density of marker phage particles (Fig. 2b). This material was also present in *in vitro* reaction

**Fig. 2** Analysis of *in vitro* products labelled with BrdUTP. **a**, A reaction mixture (125  $\mu\text{l}$ ) containing 35  $\mu\text{l}$  of extract and 10  $\mu\text{l}$  of fraction 1 from *Och11am3* infected cells and 35  $\mu\text{l}$  of extract and 10  $\mu\text{l}$  of fraction 1 from *Och12am3* infected cells was incubated for 18 min at  $30^\circ\text{C}$ . The other components of the reaction mixture were as described in the legend to Fig. 1 except that dTTP and dATP were replaced by 0.1 mM BrdUTP and 0.1 mM  $^3\text{H}$ -dATP (440 c.p.m.  $\text{pmol}^{-1}$ ) respectively. The reaction was stopped by the addition of 0.5 ml of buffer C. Ultraviolet-irradiated  $^{14}\text{C}$ -marker phage was added to the reaction mixture which was analysed by sucrose gradient centrifugation (5 to 30%, ref. 13) at 49,000 r.p.m. at  $0^\circ\text{C}$  for 50 min.  $\bullet$ ,  $^3\text{H}$ ;  $\times$ ,  $^{14}\text{C}$ . **b**, Fractions 5 to 8 of the sucrose gradient shown in (a) were pooled and 2.2 g of CsCl and 3.1 ml of Na borate buffer (50 mM sodium tetraborate, 5 mM EDTA) were added to 1.05 ml of the pooled material which was dialysed against the borate buffer. Centrifugation was performed at 33,000 r.p.m. for 44 h at  $10^\circ\text{C}$ . Fractions were collected from the bottom of the centrifuge tubes. 10  $\mu\text{l}$  of each fraction was assayed for radioactivity. The infectivity of each fraction was determined by plaque assay using *E. coli* Su20c (*Su*<sup>+</sup>) as indicator.  $\bullet$ ,  $^3\text{H}$ ;  $\times$ ,  $^{14}\text{C}$ ;  $\circ$ , infectivity (PFU per ml);  $\blacksquare$ , density ( $\text{g ml}^{-1}$ ). **c**, A reaction mixture (62.5  $\mu\text{l}$ ) containing 35  $\mu\text{l}$  of extract and 10  $\mu\text{l}$  of fraction 1 from *Och11am3* infected cells and another reaction mixture (62.5  $\mu\text{l}$ ) containing 35  $\mu\text{l}$  of extract and 10  $\mu\text{l}$  of fraction 1 from *Och12am3* infected cells were prepared as detailed in (a), and incubated separately for 18 min at  $30^\circ\text{C}$ . The reactions were stopped by adding 0.25 ml of ice-cold buffer C to each tube. Then, the two reaction mixtures were combined and centrifuged in a sucrose gradient as described in (a). Fractions 5 to 8 of the sucrose gradient were pooled and analysed by CsCl buoyant density centrifugation as described in (b).  $\times$ ,  $^{14}\text{C}$ -marker phage;  $\circ$ , infectivity (PFU per ml);  $\blacksquare$ , density ( $\text{g ml}^{-1}$ ).

mixtures in the absence of complementation (Fig. 2c). Since no material labelled *in vitro* was seen at these densities, the low density infectious material apparently existed in the infected cell extracts and might be due to unclipped phages that were recovered from infected cells in the extracts and/or fraction 1.

Several observations indicate that synthesis and packaging of  $\Phi\text{X-174}$  single-stranded DNA by our *in vitro* system proceed by mechanisms very similar to those that occur *in vivo*. Our previous results suggest that single-stranded DNA synthesis *in vitro* involves the 50S complex containing  $\sigma$  structure DNA<sup>13</sup> and that the *in vitro* 50S complex is a precursor of a particle containing circular single-stranded DNA<sup>13</sup>. We have shown here *de novo* synthesis of single-stranded viral DNA via the 50S complex by complementation experiments (Fig. 1). The synthesised single-stranded DNA is packaged in particles that are infectious and are similar in appearance to mature phage



**Fig. 3** Electron micrographs of 110S particles. Preparations of mature phages or density-labelled 110S particles (Fig. 2b) containing  $1-3 \times 10^{11}$  particles per ml were negatively stained with 2% neutral potassium phosphotungstate. **a**, Mature phages; **b**, 110S particles. Scale bar, 100 nm.



particles in the electron microscope. Further studies are required to determine the differences between 110S *in vitro* particles and 114S *in vivo* mature phages. The *in vitro* complementation system for synthesis of infectious  $\Phi$ X-174 particles described here may provide a new way of studying the interaction of viral components during phage morphogenesis.

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1. Casjins, S. & King, J. A. *Rev. Biochem.* **44**, 555-611 (1975).
2. Denhardt, D. T. *CRC Crit. Rev. Microbiol.* **4**, 161-223 (1975).
3. Dressler, D. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1934-1942 (1970).
4. Dressler, D. H. & Denhardt, D. T. *Nature* **219**, 346-351 (1968).
5. Gilbert, W. & Dressler, D. *Cold Spring Harb. Symp. quant. Biol.* **33**, 473-484 (1968).
6. Knippers, R., Razin, A., Davis, R. & Sinsheimer, R. L. *J. molec. Biol.* **45**, 237-263 (1969).
7. Sinsheimer, R. L., Knippers, R. & Komano, T. *Cold Spring Harb. Symp. quant. Biol.* **33**, 443-448 (1968).
8. Fujisawa, H. & Hayashi, M. *J. Virol.* **19**, 409-415 (1976).
9. Fujisawa, H. & Hayashi, M. *J. Virol.* **19**, 416-424 (1976).
10. Fujisawa, H. & Hayashi, M. *J. Virol.* **21**, 506-515 (1977).
11. Siden, E. J. & Hayashi, M. *J. molec. Biol.* **89**, 1-16 (1974).
12. Toneyawa, S. & Hayashi, M. *J. molec. Biol.* **48**, 219-242 (1970).
13. Mukai, R. & Hayashi, M. *J. Virol.* **22**, 619-625 (1977).
14. Funk, F. D. & Sinsheimer, R. L. *J. Virol.* **6**, 12-19 (1970).

## Extensive reverse transcription of RSV genome by nucleic acid-binding protein

THE discovery of reverse transcriptase<sup>1,2</sup> offered the possibility of using *in vitro* synthesis to study the replication of RNA tumour virus genomes in molecular detail. Attempts to study DNA provirus formation *in vitro*, however, have encountered serious obstacles: poor efficiency of transcription, small DNA products, and unequal representation of the viral RNA sequences in the DNA product have been common findings<sup>3</sup>. These difficulties in copying RNA into DNA have led to suggestions that cellular factors, such as ligases or unwinding proteins might be necessary to assist reverse transcriptase in copying the viral RNA genome into a complete DNA transcript<sup>4,5</sup>.

Binding (unwinding) proteins involved in DNA synthesis are well known in bacteria<sup>6,7</sup> and have also been described in mammalian systems<sup>8</sup>. We reported previously the isolation of a DNA binding protein from transformed chicken cells and its stimulating effect on reverse transcriptase activities<sup>5</sup>. Here we present evidence that, in the presence of the binding protein, purified reverse transcriptase from Rous sarcoma virus (RSV) synthesised an extensive, possibly complete complementary transcript of DNA from the viral RNA genome in a totally reconstituted reaction system.

RSV-transformed chicken fibroblasts were sonicated and its nucleic acids were removed by polyethylene glycol precipitation in high salt as reported previously<sup>9</sup>. The supernatant was then loaded on a single-stranded DNA-cellulose column and the binding activity eluted with 0.2 M NaCl was further passed through DEAE-cellulose and then to a CM BioGel (BioRad) column. A single peak of binding activity as measured by the membrane filtration technique<sup>9</sup> was eluted from the CM BioGel column at 0.3 M NaCl in a buffer containing 0.05 M Tris-HCl, pH 8.1, 10% glycerol, 1 mM 2-mercaptoethanol, and 1 mM EDTA. This material showed a major Coomassie blue band of over 85% homogeneity corresponding to a molecular weight of about 30,000. One microgram of the purified protein was capable of increased binding to 16  $\mu$ g of <sup>3</sup>H-poly (dA,dT) and thus at least a 500-fold enrichment in the nucleic acid binding activity was obtained from the crude sonicated extract. It had no detectable reverse transcriptase, DNA polymerase or nuclease activities when analysed<sup>10-12</sup>. Detailed isolation procedures will be published elsewhere. The binding protein was able to bind to various synthetic polynucleotides as well as to 70S <sup>3</sup>H-RSV RNA. Figure 1a and b shows the binding activity profiles when 70S <sup>3</sup>H-RSV RNA and <sup>3</sup>H-poly(A,U) were used as the substrates. The amount of binding as indicated by radioactivity retained on the membrane increased linearly until the protein saturated the binding capacity. It was calculated that at the saturation point one binding protein molecule covered 10 nucleotide bases in these substrates. Since the protein showed different rates in binding to various nucleic acids (unpublished observation), we studied the affinity of the protein to a few kinds of nucleic acid in environments of increasing ionic strength. Figure 2 shows the results of the binding study at different NaCl concentrations. There were significant decreases in binding of the protein to <sup>3</sup>H-poly A, <sup>3</sup>H-poly(A,U) and <sup>3</sup>H-poly

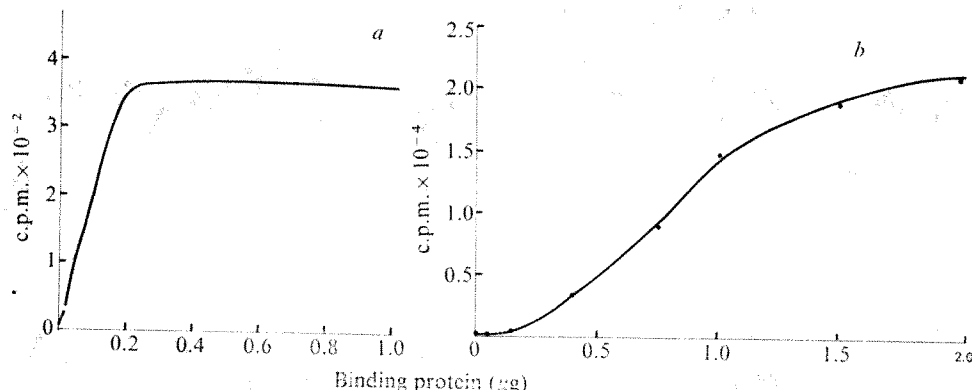
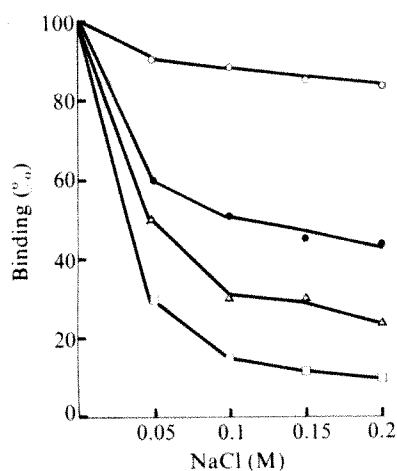
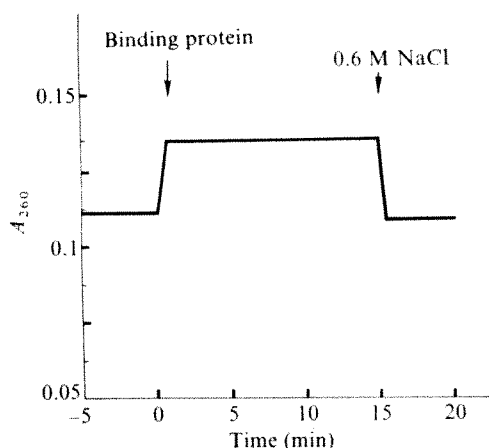


Fig. 1. Nucleic acid-binding activities of the binding protein as a function of its concentration. The assay measured the amount of radioactive nucleic acid bound to nitrocellulose filters<sup>9</sup> in the presence of the binding protein. The binding buffer contained 0.02 M Tris-HCl (pH 8.1), 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.02 M NaCl and 5% glycerol. After incubation at 37 °C for 10 min in a total volume of 0.1 ml, the reaction mixture was chilled, diluted and filtered through a Millipore nitrocellulose filter (type HA 0.45  $\mu$ m). The filter was washed with the binding buffer, dried and the radioactivity determined in a toluene-based scintillation fluid. The binding substrates: a, 70S <sup>3</sup>H-RSV RNA (0.2  $\mu$ g, 20,000 c.p.m.  $\mu$ g<sup>-1</sup>); b, poly(<sup>3</sup>H-A,U) (0.1  $\mu$ Ci; 34 mCi per mmol phosphate).



**Fig. 2** Effects of ionic strength on the binding of the protein to various nucleic acids. The buffer used during binding and washing was the same as described in Fig. 1, except that the concentration of NaCl varied as indicated. The binding in the absence of NaCl was taken as 100%. ○, 70S <sup>3</sup>H-RSV RNA (0.2 μg, 20,000 c.p.m. μg<sup>-1</sup>); ●, <sup>3</sup>H-poly A (0.1 μCi; 25 mCi; per mmol per mmol phosphate); △, poly(<sup>3</sup>H-A,U) (0.1 μCi; 34 mCi per mmol phosphate); □, <sup>3</sup>H-poly(dA, dT) (0.1 μCi; 22 mCi per mmol phosphate). Radioactive synthetic polynucleotides were obtained from Miles.

(dA,dT) when NaCl concentration was increased to 0.05 M or higher. The binding to 70S <sup>3</sup>H-RSV RNA by the protein decreased only 10% at 0.2 M NaCl, however. It is possible that some specific nucleic acid sequences in the RSV RNA have a high affinity to the binding protein and thus make the complex more resistant to dissociation in higher ionic strength. An additional piece of evidence to substantiate that the binding protein interacts with nucleic acid was provided by the following study. It is well known that the single-stranded viral RNA contains considerable secondary structures with certain regions of the genome intramolecularly hydrogen-bonded<sup>13</sup>. Therefore we studied the unwinding of this highly structured RNA by the binding protein by measuring ultraviolet hyperchromicity



**Fig. 3** Unwinding of the 70S RSV RNA by the binding protein. The reaction mixture contained 70S RSV RNA (1.6 μg) and the binding protein (15 μg) in 0.5 ml of 0.05M Tris-HCl (pH 8.1), 0.02 M NaCl, 1 mM 2-mercaptoethanol, 1 mM EDTA and 30% glycerol. Binding protein and the RNA were mixed at time zero, and  $A_{260}$  was determined with a recording Gilford Spectrophotometer at 24 °C. Solutions of the binding protein or the RNA alone in the same buffer were scanned at the same time as controls—no changes in absorbance were observed.

which is characteristic of the helix-coil transition<sup>14</sup>. The result of such an experiment is shown in Fig. 3. The absorption at 260 nm of 70S RSV RNA increased 15–20% in less than 1 min after the addition of the binding protein at 24 °C and this suggested a fast unwinding of RSV RNA by the binding protein. The hyperchromic effect was reversible on addition of NaCl to 0.6 M (Fig. 3) suggesting that the RNA was deproteinated and returned to the folded structure.

Because of the closeness of the natural primer to the 5' terminus of RSV RNA (refs 15, 16) and of the great secondary structure of the RNA, *in vitro* DNA synthesis can only proceed to a limited distance along the template and result in small DNA products<sup>5</sup>. We therefore attempted to remedy these two difficulties during reverse transcription by, first, using (dT)<sub>12-18</sub> as the primer to start DNA synthesis from the 3' terminus<sup>17</sup> and, second, by adding the binding protein to melt the hydrogen-bonded regions. DNA was synthesised in a totally reconstituted system containing purified 70S RSV RNA (ref. 18), reverse transcriptase<sup>18</sup>, dATP, dGTP, dCTP, Mg<sup>2+</sup>, <sup>32</sup>P-TTP, (dT)<sub>12-18</sub> and actinomycin D as described in Fig. 4. After incubation the reaction was stopped by EDTA and passed through a Sephadex G-50 column, DNA in the void volume was ethanol-precipitated, alkaline-hydrolysed and analysed by Agarose gel electrophoresis<sup>19</sup>. The results of the experiment are shown by autoradiography in Fig. 4. Lane 1 shows that when no binding protein was added most DNA products moved to the far end of the gel corresponding to a mobility of about 4S.

**Table 1** Hybridisation of DNA products with 70S RSV RNA

Sample	70S RSV RNA	Acid-precipitable radioactivity (c.p.m.)	Resistant fraction (%)
Control (minus S <sub>1</sub> nuclease)	—	1,120	100
Self-annealing (plus S <sub>1</sub> nuclease)	—	71	6
Hybridisation (plus S <sub>1</sub> nuclease)	+	1,066	95

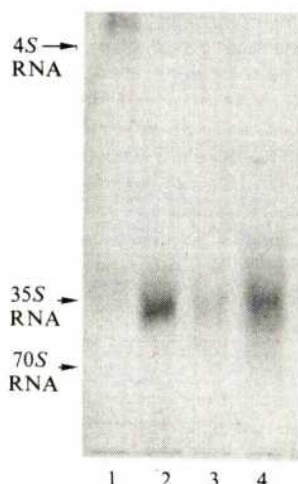
Radioactive DNA was prepared as described in Fig. 4, lane 2. DNA samples were hybridised in 2×SSC at 68 °C for 2 h in a total volume of 20 μl. When 70S RSV RNA (2 μg) was used, the ratio of RNA to DNA was estimated to be over 1,000. Resistance to S<sub>1</sub> nuclease was determined by incubating an aliquot of the incubation mixture for 2 h at 37 °C in 1 mM ZnSO<sub>4</sub>, 0.1 M sodium acetate (pH 5.0), and 2,000 units of S<sub>1</sub> nuclease, when used. The nuclease-resistant DNA was recovered as trichloroacetic acid-precipitable counts with 10 μg of salmon sperm DNA as carrier.

In lanes 2, 3 and 4 with various preparations of binding protein added, almost all of the DNA product moved much slower and its mobility corresponded to that of 35S RSV RNA. The appearance of the large products in lane 2, 3 and 4 was eliminated if the binding protein solution was first heated to 65 °C for 10 min (not shown here). To confirm that the radioactive product which corresponded to 35S in electrophoretic mobility was indeed complementary to 70S RSV RNA, we annealed the DNA product to the RNA template and digested the unreacted DNA with nuclease S<sub>1</sub> (ref. 20). Table 1 shows that 95% of radioactivity in the DNA product was resistant to the digestion after it had hybridised to 70S RSV RNA whereas DNA by itself was sensitive to the nuclease. This strongly suggested that the product was a single-stranded DNA with homology to the viral RNA genome.

The results presented here show that reverse transcriptase from RSV is able to synthesise, in a reconstructed system, a complete or nearly complete DNA copy from the purified viral RNA in the presence of the binding protein isolated

from chicken cells. There are many advantages in being able to make a complete cDNA in a totally reconstructed system. The long, representative DNA transcripts of tumour viral RNA provide a source of excellent probes for molecular hybridisation. By controlling components of the *in vitro* reaction one at a time, we can obtain a molecular detail of the mechanism of complete reverse transcription.

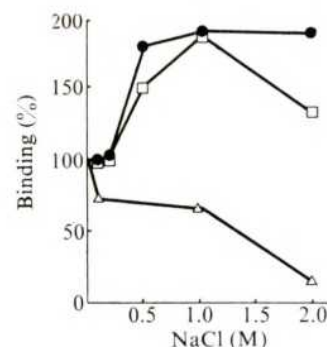
Although many investigators attempted to synthesise the complete cDNA from tumour virus RNA in a purified enzyme system, the DNA product was always considerably smaller than the template<sup>3</sup>. In some cases, there was faithful representation of the sequence information in the RNA,



**Fig. 4** Autoradiography of DNA products synthesised *in vitro* by reverse transcriptase. Incubation mixtures (0.1 ml) contained the following: 0.05 M Tris-HCl, pH 8.3, 0.06 M KCl, 5 mM MgCl<sub>2</sub>, 0.01 M dithiothreitol, 0.1 mM EDTA, 0.02% NP-40, 1.6 µg 70S RSV RNA, 0.1 µg (dT)<sub>10-18</sub>, 100 µg ml<sup>-1</sup> actinomycin D, 0.1 mM each of dATP, dCTP, dGTP, 10 µCi [ $\alpha$ -<sup>32</sup>P]TTP, (1 Ci mmol<sup>-1</sup>), 20 units of reverse transcriptase purified by the method of Grandgenett *et al.*<sup>10</sup>. The reaction mixture was incubated at 37 °C for 10 min, and then binding protein or buffer alone was added. Lane 1, buffer control. Lane 2, 4 µg of binding protein from a preparation of transformed cells. Lane 3, 4 µg from another transformed cell preparation. Lane 4, 15 µg of binding protein from non-transformed cells. Incubation was carried out further at 24 °C for 4 h. At the end of the incubation, the reaction mixture was adjusted to contain 0.05 M EDTA, 0.2% sodium sarkosyl and 50 µg yeast RNA. The DNA product was isolated by Sephadex filtration and alkali hydrolysis according to Rothenberg and Baltimore<sup>19</sup>. The samples thus obtained were divided into two parts, one for electrophoresis followed by autoradiography and the other, for hybridisation studies. Electrophoresis of the DNA products (approximately 1,000 c.p.m. per slot) was done in 0.8% Agarose gel (10 cm long) for 3.5 h at 100 V (ref. 25). Non-radioactive 70S RSV RNA, 35S RSV RNA obtained by heating and chilling of 70S (ref. 26) and tRNA were co-electrophoresed. Ethidium bromide was used to stain these markers after the electrophoresis and arrows indicate their positions. The gel was dried and autoradiographed as described by Maizel *et al.*<sup>27</sup>.

but only a small portion of the template was represented in a greatly disproportionated amount<sup>19,21,22</sup>. The incomplete cDNA transcripts of the RSV RNA occurred as discrete size classes rather than a continuous array of decreasing sizes<sup>23</sup>. This suggested that when reverse transcriptase started DNA synthesis at the primer and transcribed along the single-stranded RNA, it could encounter hairpin loops or other forms of secondary structure present in the viral RNA at one or more points, thereby resulting in a partial blockage of transcription or in dissociation of the enzyme at these discrete sites. The nucleic acid-binding protein, by virtue of its ability to bind and unwind the RNA molecule

(Figs 1 and 3), can hold the RNA in a rigid, extended conformation which affords a favourable configuration for reverse transcriptase to continue the cDNA synthesis instead of coming to a stop at the hairpin loops. We believe that the affinity of the binding protein to RSV RNA is such that when reverse transcriptase moves along the template, it replaces the binding protein to continue cDNA synthesis. This was supported by evidence shown in Fig. 5 that reverse



**Fig. 5** Effect of ionic strength on the binding of reverse transcriptase to various nucleic acids. The buffer used during binding and washing was the same as described in Fig. 1, except that the concentration of NaCl varied as indicated. Binding of reverse transcriptase (5 units) to the substrate in the absence of NaCl was taken as 100%. ●, 70S <sup>3</sup>H-RSV RNA (0.2 µg; 20,000 c.p.m. µg<sup>-1</sup>); ×, <sup>3</sup>H-poly U (0.1 µCi; 30 mCi per mmol phosphate); △, poly(<sup>3</sup>H-A, U) (0.1 µCi; 34 mCi per mmol phosphate).

transcriptase bound to RSV RNA much tighter than the binding protein. In this experiment, the binding of reverse transcriptase to 70S RSV RNA increased twice at ionic strengths as high as 1.0 M NaCl, in contrast to a gradual dissociation of the binding protein from RSV RNA at higher ionic strength (Fig. 2).

Although the binding protein reported here was purified from RSV-transformed chick cells, the protein did not seem to be coded by the virus. In a preliminary study we also detected a binding protein of similar properties in normal chicken cells (Fig. 4, lane 4). A viral protein binding tightly to the viral RNA has been recently found in the RSV viral core<sup>24</sup>. This viral core protein formed such a tight complex with the viral RNA that it inhibited rather than stimulated reverse transcription (J. Leis, personal communication).

We are now characterising the DNA product in more detail. It will be of great interest to synthesise a DNA duplex (provirus) of the whole viral genome and study its infectivity as well as nucleotide sequences of the fragments obtained from restriction enzymes.

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1. Baltimore, D. *Nature* **226**, 1209-1211 (1970).
2. Temin, H. M. & Mizutani, S. *Nature* **226**, 1211-1213 (1970).
3. Green, M. & Gerard, S. F. *Prog. Nucleic Acids Res. molec. Biol.* **14**, 187-334 (1974).
4. Temin, H. M. & Baltimore, D. *Adv. Virus Res.* **17**, 129-186 (1972).
5. Hung, P. P. & Lee, S. G. *Nature* **259**, 499-502 (1976).
6. Sigal, N., Dalius, H., Kornberg, T., Gefter, M. L. & Alberts, B. M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3534-3541 (1972).
7. Alberts, B. M. & Frey, L. *Nature* **227**, 1313-1318 (1970).
8. Herrick, G. & Alberts, B. W. *J. biol. Chem.* **251**, 2124-2132 (1976).
9. Tsai, R. L. & Green, H. *J. molec. Biol.* **73**, 307-316 (1973).
10. Gradgenett, D. P., Gerard, G. F. & Green, M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 230-234 (1973).
11. Aposhian, H. V. & Kornberg, A. *J. biol. Chem.* **237**, 516-525 (1962).
12. Holloman, W. K. & Holliday, R. *J. biol. Chem.* **248**, 8107-8113 (1973).
13. Delius, H., Duesberg, P. H. & Mangel, W. F. *Cold Spring Harb. Symp. quant. Biol.* **39**, 835-843 (1975).



14. Marmur, J. & Doty, P. *Nature* **183**, 1427–1429 (1959).
15. Staskus, K. A., Colletti, M. S. & Faras, A. J. *Virology* **71**, 162–168 (1976).
16. Taylor, J. M. & Illmensee, R. J. *Virology* **16**, 553–558 (1975).
17. Taylor, J. M. *et al. Biochemistry* **12**, 460–467 (1973).
18. Robinson, W. S., Robinson, H. L. & Duesberg, P. H. *Proc. natn. Acad. Sci. U.S.A.* **58**, 825–834 (1967).
19. Rothenberg, E. & Baltimore, D. J. *Virology* **21**, 168–178 (1977).
20. Ando, T. *Biochim. biophys. Acta* **114**, 158–168 (1966).
21. Junghans, P., Duesberg, P. H. & Knight, C. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4895–4899 (1975).
22. Collett, M. S. & Faras, A. J. *J. Virol.* **16**, 1220–1228 (1975).
23. Haseltine, W. A., Kleid, D. G., Panet, A., Rothenberg, E. & Baltimore, D. *J. molec. Biol.* **106**, 109–131 (1976).
24. Sen, A., Sherr, C. J. & Todaro, G. J. *Cell* **7**, 21–32 (1976).
25. Adesnik, M. *Meth. Virol.* **5**, 125–177 (1971).
26. Canaan, E. & Duesberg, P. J. *Virology* **10**, 23–31 (1972).
27. Maizel, J. V. Jr. *Meth. Virol.* **5**, 179–245 (1971).

## 2-Aminoadenine is an adenine substituting for a base in S-2L cyanophage DNA

THE properties of DNA from viruses acting on blue-green algae (cyanophages) have been studied in only a few instances<sup>1,2</sup>. It is not known whether these DNA molecules contain any unusual bases<sup>3–7</sup> or some modified (methylated) bases known to be present in DNA of many prokaryotes and to be specific for host modification and restriction phenomena. Here we report the isolation and identification of the base 2-aminoadenine and its deoxyribonucleoside from S-2L cyanophage DNA. Some unusual properties of this DNA in which adenine is completely substituted with 2-aminoadenine are described.

Cyanophage S-2L was isolated from water samples taken in the outskirts of Leningrad. It can lyse blue-green algae *Synechococcus* sp. 698 and *S. elongatus* strains 58 and 6907 (from the collection of the Biology Institute, Leningrad State University). The phage was grown on *S. sp.* 698 and concentrated by chromatography on DEAE-cellulose (Whatman DE-32) and purified by centrifugation in CsCl gradient<sup>2,8</sup>. The phage consists of an icosahedral head, 56 nm in diameter, with a flexible non-contractile tail 120 nm long and 10 nm thick. S-2L phage is morphologically similar to SM-2 cyanophage<sup>9</sup>.

The nucleic acid of phage S-2L is linear double-stranded DNA. It reacts typically for deoxyribose with a diphenylamine-containing reagent according to Dische; it is not hydrolysed in

alkali (0.7 M NaOH, 18 h at 37 °C) or with pancreatic RNase. The mean contour length of S-2L DNA molecules prepared according to Kleinschmidt<sup>10</sup> was  $14.4 \pm 0.5 \mu\text{m}$ , corresponding to a molecular weight<sup>11</sup> of  $28 \pm 0.5 \times 10^6$ . The average value for  $S_{20,w}^{0}$  for S-2L DNA was  $32.5 \pm 0.3 S$ —a molecular weight value<sup>12</sup> of  $26 \pm 1 \times 10^6$ .

The buoyant density of S-2L DNA, relative to *Escherichia coli* B DNA ( $\rho = 1.7109 \text{ g cm}^{-3}$ ) was found to be  $1.731 \text{ g cm}^{-3}$ . When S-2L DNA was centrifuged with *Micrococcus lysodeikticus* DNA ( $\rho = 1.731 \text{ g cm}^{-3}$ ), a single symmetric ultraviolet absorbing peak was observed.

The hyperchromic value of S-2L DNA at 260 nm is 27–29%, at 280 nm it reaches 50% (Fig. 1);  $T_m$  in 0.1 SSC is 85.6 °C; in 0.01 M sodium phosphate buffer pH 7.0, 0.001 M Na<sub>2</sub> EDTA  $T_m$  is 84.3 °C. The DNA melts within a small temperature range ( $\Delta T_m = 3^\circ\text{C}$ ) which is typical of homogenous viral DNA. A characteristic feature of native S-2L DNA is the unusually low spectral ratio ( $A_{\text{max}}/A_{\text{min}} = 1.6$ ,  $A_{260}/A_{230} = 1.5$ ,  $A_{260}/A_{280} = 1.75$ ).

In contrast to the circular dichroism (CD) spectrum of usual natural DNA that possesses a single positive band at 276 nm, S-2L DNA has two almost equal positive bands, at 290 nm and 265 nm, (Fig. 2). This very characteristic CD spectrum and the other unusual ultraviolet spectral properties strongly suggest the presence of some unusual base in S-2L phage DNA.

Fig. 1 S-2L phage DNA absorption spectrum in 0.1 SSC; a, at 20 °C and b, at 90 °C.

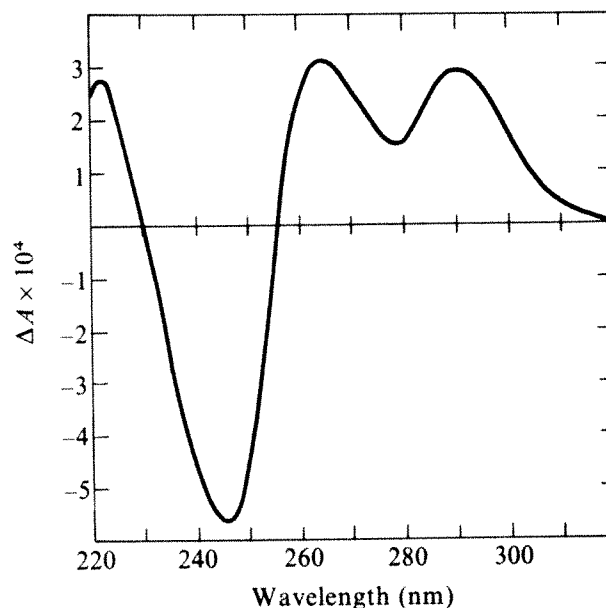
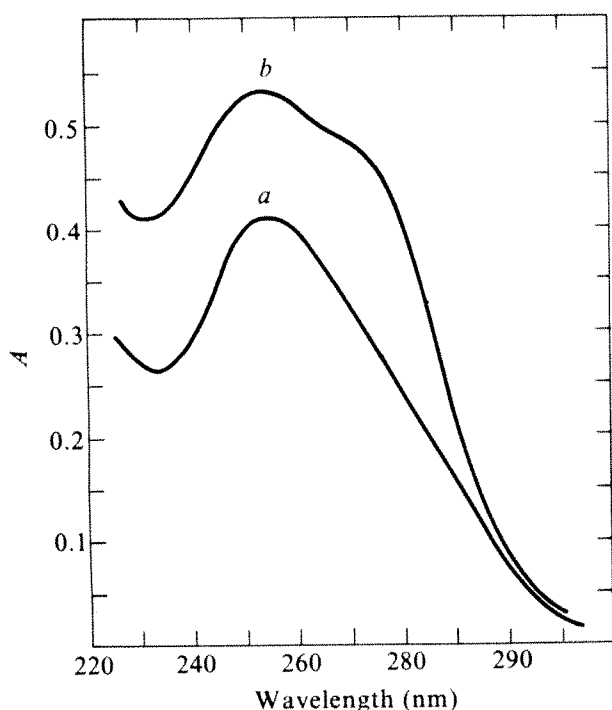


Fig. 2 CD spectrum of S-2L phage DNA in 0.01 M phosphate buffer, pH 7.0–0.001 M Na<sub>2</sub>-EDTA (1.3 absorbance units at 260 nm per ml).

For determination of the base composition, the DNA was hydrolysed into bases by 57% HClO<sub>4</sub> (100 °C, 1 h) or 99% HCOOH (176 °C, 1 h). Bases were separated by thin layer chromatography (TLC) (Fixion 50 × 8 cation exchanger (Chinoin) in 2.8 M HCl)<sup>13</sup>; as well as thymine, cytosine and guanine, a fourth base with the lowest  $R_f$  value, was present. This base was purified by TLC on cellulose (Filtrak) in different solvent systems. The isolated base shows intensive blue fluorescence in ultraviolet light and a characteristic ultraviolet adsorption spectrum (two maxima); the base has the same spectral (Fig. 3, Table 1) and chromatographic properties as authentic 2-aminoadenine (Chemapol). On bromination the base degrades—like 2-aminoadenine and unlike adenine (Fig. 3).

Complete enzymatic hydrolysis of S-2L DNA to nucleosides, yielded 2-aminoadenine deoxyribonucleoside after two-dimensional TLC of deoxyribonucleosides on cellulose<sup>14</sup>. The ultraviolet and fluorescence spectra of the 2-aminoadenine deoxyribonucleoside isolated were identical to those reported for



**Table 1** Spectral properties of the novel base and deoxyribonucleoside from cyanophage S-2L DNA compared with synthetic 2-aminoadenine

Spectral parameters	Phosphate buffer 0.05 M pH 6.7		Spectral properties in			
	Deoxyribonucleoside*	Base†	0.1 M HCl 2-Aminoadenine treated as base from phage DNA†	2-Aminoadenine	0.1 M KOH Base†	2-Aminoadenine
$\lambda_{\max}(1)$ , (nm)	254	241.5	241.5	241	Inflection (243 nm) 283	Inflection (243 nm) 283
$\lambda_{\max}(2)$ , (nm)	279	282	282	281.5	—	—
$\lambda_{\min}(1)$ , (nm)	233	231.5	231.5	231.5	—	—
$\lambda_{\min}(2)$ , (nm)	263	261.5	261.5	261.5	258	257
$A_{\max}(1)/A_{\min}(2)$	0.12	1.75	1.75	1.80	—	—
$A_{\max}(2)/A_{\min}(2)$	0.13	1.86	1.86	1.90	2.46	2.42
$A_{230}/A_{260}$	0.72	1.45	1.60	1.55	—	—
$A_{240}/A_{260}$	0.72	1.73	1.80	1.80	1.32	1.26
$A_{250}/A_{260}$	0.12	1.50	1.50	1.30	1.25	1.10
$A_{270}/A_{260}$	0.11	1.30	1.30	1.45	1.39	1.55
$A_{280}/A_{260}$	1.29	1.84	1.85	1.89	2.40	2.38
$A_{290}/A_{260}$	0.88	1.51	1.50	1.53	2.20	2.10
Excitation maximum in 0.05 M K phosphate buffer ( $\lambda$ nm)	300	300	300	300	—	—
Fluorescence maximum ( $\lambda$ , nm)	360	360	360	—	—	—

\*Isolated from hydrolysed phage DNA by two-dimensional TLC on cellulose (Filtrak)<sup>14</sup>.

†Isolated by TLC on Fixion 50 × 8 in 2.8 M HCl<sup>13</sup>, repeated TLC on cellulose (Filtrak) in isopropanol–11 M HCl–H<sub>2</sub>O (170:45:35 v/v) and then repeated TLC in the solvent systems *n*-butanol–H<sub>2</sub>O–25% NH<sub>4</sub>OH (60:10:0.1 v/v); isopropanol–H<sub>2</sub>O (70:30), NH<sub>3</sub> in gas phase; *n*-butanol–5 M acetic acid (2:1 v/v).

2-aminoadenosine<sup>15</sup>. Thus, S-2L phage DNA contains 2-aminoadenine, guanine, cytosine and thymine; no other bases were found. DNA of host alga *Synechococcus* sp. 698 contains usual bases, including adenine, rather than 2-aminoadenine, and its GC content is 70.0 mol %.

On complete enzymatic hydrolysis the S-2L DNA was found to contain (mol %): 2-aminoadenine deoxyribonucleoside, 15.9 ± 0.5; deoxythymidine, 15.4 ± 0.1; deoxycytidine, 34.4 ± 0.4 and deoxyguanosine, 34.3 ± 0.3. This DNA thus contains equimolar quantities of 2-aminoadenine and thymine and of guanine and cytosine.

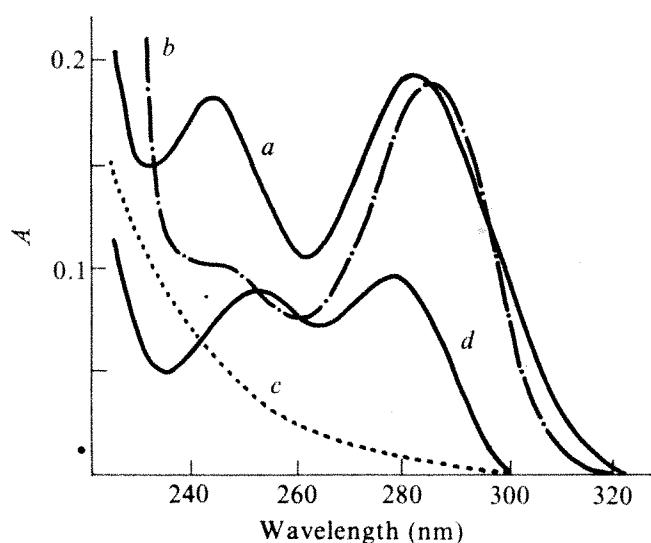
The presence of the additional amino group in 2-aminoadenine as compared to adenine permits formation of the three hydrogen bonds in 2-aminoadenine–thymine base pair<sup>16</sup>. This seems to account for the higher  $T_m$  (85.6 °C, in 0.1 × SSC) for 2-aminoadenine containing S-2L DNA as compared with usual adenine-containing DNA ( $T_m$  = 82.0 °C) which is equivalent to

the former in GC content. According to the  $T_m$  value, S-2L DNA should contain 77.4 mol % of GC, since from the  $\rho$  value (1.731 g cm<sup>-3</sup>) the GC content should be 72.5 mol %<sup>17,18</sup>; in fact the DNA contains only 68.7 mol % of GC.

The anomalous CD spectrum of S-2L DNA is similar to the CD spectrum of polyribodiaminopurine–poly U complex<sup>19</sup>, the difference being only in the sign of the bands at 290 nm. Dichroism measurements may therefore prove useful for the possible detection of 2-aminoadenine in other viral nucleic acids.

In contrast to other viruses containing novel bases substituting for ordinary pyrimidines<sup>3–7</sup>, S-2L phage seems to be the first case in which one of the ordinary purine bases in DNA is completely substituted with a modified base. It seems likely that 2-aminoadenine incorporates into S-2L phage DNA as a ready-made nucleotide, rather than forming at the polynucleotide level as a result of amination of adenine residues in DNA. In cells of virus-infected blue-green algae new paths for the synthesis of the adenine nucleotide derivative may therefore exist: a special system for regulation and discrimination between the replication of host and unusual 2-aminoadenine-containing viral DNA would be needed.

**Fig. 3** Ultraviolet spectra of the base and its deoxyribonucleoside isolated from S-2L phage DNA. *a*, Base in 0.1 M HCl; *b*, base in 0.1 M KOH; *c*, base after bromination; *d*, deoxyribonucleoside in 0.05 M K-phosphate buffer, pH 6.7.



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1. Padan, E. & Shilo, M. *Bact. Rev.* **37**, 343–370 (1973).
2. Adolph, K. W. & Haselkorn, R. *Virology* **54**, 230–236 (1973).
3. Wyatt, G. R. & Cohen, S. S. *Biochem. J.* **55**, 774–782 (1953).
4. Kallen, R. G., Simon, M. & Marmur, J. *J. molec. Biol.* **5**, 248–250 (1962).
5. Takahashi, I. & Marmur, J. *Nature* **197**, 794–795 (1963).
6. Kuo, T. T., Huang, T. C. & Teng, M. H. *J. molec. Biol.* **34**, 373–375 (1968).
7. Vanyushin, B. F., Belyaeva, N. N., Kokurina, N. A., Stelmashuk, V. Y. & Tikhonenko, A. S. *Molekularnaya biologiya* **4**, 724–729 (1970).
8. Brunk, C. F. & Leick, V. *Biochim. biophys. Acta* **179**, 136–144 (1969).
9. Fox, J. A., Booth, S. J. & Martin, E. L. *Virology* **73**, 557–560 (1976).
10. Kleinschmidt, A. K. *Meth. Enzym.* **12B**, 361–379 (1968).
11. Wilkins, M. H. F. *Science* **140**, 941–950 (1963).
12. Freifelder, D. *J. molec. Biol.* **54**, 567–577 (1970).
13. Tomasz, J. *Analyt. Biochem.* **68**, 226–229 (1975).
14. Felix, F., Potter, J. L. & Laskowski, H. *J. biol. Chem.* **235**, 1150–1153 (1960).
15. Davol, J. & Lowy, B. A. *J. Am. chem. Soc.* **73**, 1650–1655 (1951).
16. Cerami, A., Reich, E., Ward, D. C. & Goldberg, I. H. *Proc. natn. acad. Sci. U.S.A.* **57**, 1036–1042 (1967).
17. Marmur, J. & Doty, P. *J. molec. Biol.* **5**, 109–118 (1962).
18. Schildkraut, C. L., Marmur, J. & Doty, P. *J. molec. Biol.* **4**, 430–443 (1962).
19. Howard, F. B., Fraizer, J. & Miles, H. T. *Biochemistry* **15**, 3783–3795 (1976).

# reviews

## Tectonic changes in global terms

D. H. Tarling

*The Evolving Continents.* By Brian F. Windley. Pp. xviii + 385. (Wiley: London and New York, 1977.) £14; \$28.

THIS is, without doubt, one of the best of recent books concerned with the Earth's evolution and is the first which really does attempt to see tectonic changes in terms of the total history of the Earth. At last, the Precambrian has been treated as an integral part of the Earth instead of being confined either to specialist books or to forming the odd paragraph added on to an evolutionary story that is otherwise entirely concerned with the Phanerozoic. It would be ingenuous to fault different parts of the book for lack of sufficient data or for inadequate coverage of general ideas, and any such criticism must be considered secondary to the real achievement of combining an optimum of both information and ideas within less than 400 pages.

The level is essentially for third year undergraduates and upwards and should form a standard advanced text, following an Arthur Holmes introduction to the earth sciences, although some amplification of plate tectonic terminology will be needed—lithosphere is, for example, not defined, but it is certainly useful to have the Burke-Dewey "aulacogen" described fully, as this term is still not in most dictionaries. The lack of definition of lithosphere compared with that for aulacogen, in fact, reflects the essentially geological-geochemical approach throughout, with the geophysics largely restricted to palaeomagnetism. Nonetheless, to complain about this lack of geophysical content is, to some extent, unfair, as sufficient books have appeared recently on geophysical plate tectonics in the Phanerozoic to sate most people's appetites.

Precambrian geology and models comprise half of the book with the Phanerozoic and generalities forming the remaining half. Logically, it starts with the Archaean, with one chapter on high grade terranes; one on low grade; and a third is concerned with models of Archaean crustal evolution. The Proterozoic takes a little longer, with four chapters preceding the summary of crustal evolutionary models for this period. The ability to condense the Archaean better than the Proterozoic

possibly reflects Windley's previous background, but it must be stressed that meaningful generalisations about the evolution of Proterozoic regimes have previously been virtually restricted to the publications of Sutton and Watson.

Naturally, such an initial compilation of information and ideas can be criticised in numerous places, although Windley often plays safe by outlining other people's descriptions without significant critical comment. This is understandable in relation to 'factual' observations, but is less satisfactory where ideas are presented and discussed. Albeit rarely, Windley's views sometimes tend to bias the presentation of other schema. Criticism is always possible on the detail of the coverage—half-a-page on the West Australian Kalgoorlie System is barely sufficient to provide references; and this is virtually all it does. But any real extensions would have doubled the size of the book and probably more than doubled its price.

The second half, dealing with the generally accepted Phanerozoic plate tectonics, is less successful, and it is arguable as to the extent to which it could be both reduced and expanded. There are three chapters on orogenic belts—Caledonian, Hercynian and Alpine—which illustrate this point. The Caledonian chapter could well be reduced, as this is described well elsewhere. The Alpine chapter is so abbreviated as to be virtually notes against which a specialist could view his own area or interest, but they are too skeletal for the undergraduate. The chapter on Hercynian is sufficiently short to reflect the surprising lack of interest by most geologists in this period, but does not adequately represent the multiplicity of views which interested earth scientists have expressed. All three, as usual, also suffer from a parochial 'Laurentian' view, with no consideration of the extension of such systems into, for example, Asia.

The second half also suffers from an apparent desire to insert as many aspects as possible. It is a pity, for example, that the elementary outline of palaeomagnetism is not severely reduced to provide space for a discussion of how isotopic studies actually

indicate the source for volcanics, as this knowledge is virtually assumed in the opening chapters. Similarly, the treatment of palaeoclimates is so elementary as to be almost unnecessary. Also crammed within this half are the chapters on seafloor spreading, cordilleran mountain chains, palaeontological implications, island arc systems and an outline of the breakup of Pangaea. Some of these chapters tend to side-track, so that the breakup of Pangaea seems to be mostly related to the evolution of volcanic systems rather than the actual history of break up and separation which is again essentially in note form.

One of the other new features of the book is an attempt to introduce sections of the characteristic mineralisation associated with specific times and localities. This aspect clearly reflects the general, increased interest in the applicability of academic research to real problems. Windley is to be congratulated on his attempt, but this is, unfortunately, probably the least satisfactory part—even though this must be considered a major achievement. The problem is probably one of space, as most of the mineralisation sections are fairly bald statements of what minerals occur where, but with inadequate discussion of why they occur in those particular places at those particular times. To some extent such origins may still be unknown and would therefore be highly speculative, but it is disappointing to find that possible causative factors are not adequately discussed. The same is even more true for the appraisal of the changing tectonic processes. Drastically different tectonic regimes are described for the Archaean, Proterozoic and Phanerozoic, but the only causative feature which is described as being different is that of radiogenic heat production within the Earth. This change in heat production with time is quantified, but Windley does not offer any considerations for the implication of such changes to, for example, lithospheric thickness and the major, almost catastrophic, changes in the physical behaviour of the lithospheric plates which occur as such changes took place. In other words, there is really no discussion of the actual mechanisms—discussions of con-

vection are mostly restricted to minor Archaean problems. On this basis, the book tends to be overly concerned with the end-products of mantle processes and virtually ignores the mechanisms by which they are derived, and therefore why their operation may have changed with time. Some discussion of the vertical variation in the continental crust is also required and the way in which these differences have evolved should have been discussed. Reducing some of the side-lines in the second half and really considering the changing mechanism of tectonic change would then have been more consistent with the title.

The production is excellent and the illustrations are ample; in fact almost too many, as several could have been usefully combined. This tends to apply also to the text, where Windley's desire to give other people's views possibly

would have been better presented as a clear statement with references. These features tend to make some parts seem rather too much like lecture notes, with slides of published work, than is desirable. Such comments are, however, intended sympathetically, as it would be hard to improve the text without grossly expanding the total length. Nonetheless, although improvements are possible, particularly in considering the mechanism of tectonic change, this book can be unreservedly recommended to final-year and postgraduate students both as a textbook and a source of references for further studies. It must be an essential on any final-year earth science reading list. □

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## Dynamic systems

*Synergetics: An Introduction. Non-Equilibrium Phase Transitions and Self-Organisation in Physics, Chemistry and Biology. By H. Haken. Pp. xii+325. (Springer: Berlin and New York, 1977.) DM72; \$31.70.*

FOR some time now, it has been the main concern of the author to popularise the subject he has chosen to call "synergetics". To most readers, however, this term is less familiar than the subject itself—the theory of phase transitions in co-operative dynamic systems, applied to examples ranging from physics to sociology. Part of the material presented in this book is already contained in several review articles and conference proceedings, but now H. Haken presents in this volume a detailed and comprehensive account of the basic principles and applications of this "interdisciplinary field of research".

The term "synergetics" was prompted by the variety of dynamic systems stemming from physics, chemistry, biology and sociology which show striking similarities at their points of instability. As the book is meant to be a student textbook, the author provides in the first few chapters the mathematical prerequisites. After some introductory sections on probability and information theory, the reader becomes acquainted with stochastic processes (Brownian motion, random walks and their description by Master equations) and deterministic motion (here, the concepts of stability, critical points, hard and soft mode excitations, limit cycles, and so on, are introduced).

The connection between deterministic and stochastic processes (or chance and necessity) is provided by the Fokker-Planck equation approach, which is discussed extensively and used to introduce the Landau theory of phase transitions. Combining all these concepts, the author then explains in a rather formal and abstract way the generation of order by self-organisation. Then, separately, these formal considerations are applied to various examples. Starting from physics (laser and hydrodynamic instabilities), the reader is led to nonlinear chemical reactions (with their showpieces, Brusselator and Oregonator) and biological systems (models for ecology, evolution and morphogenesis); and, finally, a model for interacting social groups is presented.

This book certainly serves its purpose as an introductory student text, as it requires little previous knowledge. Besides tradition (synergetics is not part of a usual university curriculum), the only obstacle to its wide distribution among students may be its fairly high price.

Experienced readers will certainly profit from the variety of examples presented and the rather detailed reference section, a good guide to the topical literature. It is a pity, however, that some of the important work of several Japanese groups on nonlinear chemical reactions is missing—instead, the author has chosen to cite unpublished work carried out by his own group. But this minor weakness is by far outweighed by many advantages.

**R. Schraner**

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## Quantum mechanics

*The Uncertainty Principle and the Foundations of Quantum Mechanics. Edited by W. C. Price and S. S. Chissick. Pp. 572. (Wiley-Interscience: London and New York, 1977.) £19.50; \$38.*

BEING a collection of articles, this book cannot be quite faithful to its title: particularly since it is just one book in a series of books, each designed as a tribute to one of the founders of modern physics. Clearly, such publications are not intended to provide anything like a systematic and critical survey of a subject, especially when the latter is highly structured, as is the case with regard to the foundations of a discipline. But what such books can do is to present significant partial results and to direct the reader's attention toward recent specialised research. This is in fact what is achieved, and in a highly satisfactory way, by this book.

Although I benefited from several of the valuable articles the book contains, let me follow my own taste in selecting just one for review: the contribution by G. Lanz. This paper bears on what is often considered to be the only problem remaining in the foundations of quantum mechanics, namely the problem of measurement. Its description of the difficulties besetting that problem is clear. So is its classification of the two conceptions of quantum mechanics that (barring non-local hidden variables) one may still discuss quantum mechanics as the basic theory of physics or quantum mechanics as a mere theory of microsystems, anchored on a (still to be created) theory of macrosystems. The author considers that the first view should be dismissed because of insuperable difficulties with the problem of measurement. As for the second conception, it obviously requires a theory of macrosystems, and an unambiguous definition of what a macrosystem is.

Lanz acknowledges that he has until now no completely definite answers to such questions. He sketches, however (with some profusion of mathematical detail), an approach based on Ludwig's formalism which he believes is promising. It involves a limiting procedure the physical meaning of which is unfortunately not discussed. The general idea is to extract from the N-body theory a part that could be used for a theory of macrosystems; the rest could then be thrown away as physically irrelevant. An interesting but perhaps premature question is whether the future theory will be able to define macrosystems in such a way that at least some finite (and really existing) systems are macroscopic.

**Bernard d'Espagnat**

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## Outlines of Mercury

*The Atlas of Mercury.* By Charles A. Cross and Patrick Moore. Pp. 48. (Mitchell Beazley: London; Crown New York, 1977.) £5.95: \$10.

JUDGING by the presence of the word "telescope" in the glossary at the back of this book, Cross and Moore are attempting a relatively popular treatment of their subject. To this end, in addition to the glossary, they provide helpful diagrams to explain anything that might give pause for thought; for example, why Mercury has a day which is two of its years long. This consideration, shown also in the innumerable illustrations and photographs provided, makes the book easy and informative reading for the relatively uninitiated; whereas, on the other hand, its comprehensive nature should mean that it is not without interest for more advanced readers.

The broad scope of the book also provides a neat way of illustrating just how much of our present knowledge of Mercury we owe to that triumph of American technology, Mariner 10, since the whole history of observation (including much duplication) up to the time of Mariner 10, takes up far less of the book than the results gained from that one space-probe. Those who do not have the time to count the relevant number of pages need only compare Chapman's pre-mission map of the planet (p5) with the map drawn by Cross. It ought perhaps to be remarked, however, that Chapman's map is the upper of the two described on p5 and that it is not the latest pre-Mariner map; this distinction belonging to a map drawn by J. B. Murray *et al.* (*Icarus*, 17, 576-584; 1972). Cross's excellent maps, which form the basis of this book, are centred on the useful subsolar point, and the accompanying photographs give an accurate idea of the planet's surface, perhaps the only omission being a high resolution picture of the Hilly and Lineated Terrain. Also, some mention of the processing these pictures have undergone might have been interesting.

The text accompanying the pictures, however, is the weakest part of the book. There was just not enough geological nitty-gritty to maintain my interest in the endless succession of craters; and calling them circular "enclosures", "formations" or "walled plains" did not help. It could be said that this is the criticism of a geologist, and rather unfair, since the book is an elementary one wherein the authors are clearly trying to keep things simple, but the approach of describing things by quadrants and vast numbers of photographs rather than subjects has provided gaps for the astronomy-orient-

ated authors to stray into, and go astray in, the geological field. For example, on p24, it is argued that, because of the lower number of scarps and the "various younger craters and basins", the area covered by the South-West Quadrant is younger on the whole than the South-East Quadrant. In fact, it is the oldest features which indicate the age of the terrain and in terms of age and distribution of craters, the South-West Quadrant could be older, if anything, than the South-East, but whether it is or not, the statement would have been unnecessary in the first place if something more solid had not been cut out by the simplification process.

A key to the age of the surface is the nature of the Intercrater Plains, which on p18 are correctly described as the regions between the main craters (assuming there is nothing else there, such as Smooth Plains). In the next paragraph, however, it is stated that there is an "obvious" resemblance between this intercrater unit and the highlands of the Moon, which for the most part are nothing but craters. The original description (Trask and Guest, *J. Geophys. Res.*, 80, (17), 2461-2477; 1975) is quite clear: "Intercrater Plains (unit) has a closely similar analog on the Moon in the Pre-Imbrian Plains of Wilhelms and McCauley (*Miscellaneous Geological Investigation Map* 1-703, US Geological Survey; 1971)". That is to say, the Intercrater Plains are similar morphologically to terrain situated between the main craters of a relatively sparsely cratered unit within the southern highlands of the Moon. The lunar unit is of much smaller extent and for this reason, among others, is not thought to originate in the same way.

On p30, a prominent ray is said to be "clearly a northward extension of Heemskerck Rupes". If this is so, then it is an exciting discovery linking what has hitherto been considered as a superficial unit, thrown out by the impact of a meteorite, to a feature interpreted as a very large compressional fault scarp in the planet's crust. I am not excited. On p35 Rupes Zeehan is said to be formed from wall remnants of pre-Caloris craters. Certainly, it seems to be influenced by them, but faces the wrong way for the authors' description to be correct.

On p21, it is indicated that the large scarp in the crater Po Ya "seems to be" a lava front rather than a compressional feature. There are good examples of *small* scarps apparently confined to the Smooth Plains (not present in Po Ya) on some crater floors which some think might be the fronts of highly viscous lava flows (c.f. highly fluid mare basalts). The Po Ya scarp is not one of these. This error is an example of the heavy volcanic bias present in the book. Thus, it is thoroughly misleading of the authors to suggest that there is any controversy over

the cause of the craters on the Moon (and therefore Mercury), even if they do plump for a mixture of both impact and volcanic hypotheses. The vast majority of craters on the Moon, Mars and Mercury were caused by the impact of impinging space debris and because they are morphologically different from the volcanic features also present on the Moon and Mars (but not yet pointed out with any confidence on Mercury), there is no argument as to their origin.

If all this is not entirely compatible with the implication in the proud claim on the cover that this is "the most concise and accurate account of the planet available" (Sir Bernard Lovell), it is also true that, although the mistakes tend to mislead or confuse as to certain pictures or features, they do not seriously misrepresent the fundamental processes which have shaped the planet's surface. In addition, apart from the volcanic bias, the authors do strive throughout to maintain an admirable scientific restraint. For example, the attractive theory that Mercury was once a satellite of Venus is quite properly described as "still highly speculative". On the whole then, this book can be commended to the readers for whom it was written. **W. P. O'Donnell**

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## Satellite technology

*The Versatile Satellite.* By Richard W. Porter. Pp. +173 (Oxford University: Oxford and London, 1977.) £4.95.

THE author sets himself the task of explaining "what a satellite is, how it is put into orbit (and why it does not promptly fall down again), what special considerations enter into its design, and especially what it is 'good for' and why". Since all of this is attempted in 173 pages, it is not surprising that the reviewer, an environmental scientist, found the treatment of environmental applications was rather superficial. Nevertheless, a great deal of technical information is conveyed in a clear and concise manner.

The book is well structured, beginning with the history of launching the satellites and their evolution from German and Russian rocketry. This is followed by a useful explanation of orbits and energy requirements for different types of satellite configurations. In a discussion of design requirements, emphasis is placed on the special circumstances of lack of atmosphere, absence of gravity, ionosphere, thermal environment, electromagnetic radiation, geomagnetic field, charged particle environment, and micro-meteoroids. The author praises the satellite designer for overcoming this unfriendly satellite environment within limitations of weight and cost. However, the cost-benefit aspects of the satellite programmes are not discussed in depth in this book.

The environmental scientist should have particular interest in those chapters explaining "what it is good for and why". These discuss applications in the fields of communications; weather forecasting and monitoring; navigation; surveying the oceans and land; monitoring flora, fauna and fish; and spaceborne laboratory experiments.

The impressive growth of satellite communications is well documented and the characteristics of Intelsat satellites are described in some detail. The technologist and engineer may revel in the details of stabilisation, antennas and electronic systems, but all readers will flinch in the face of space jargon — for example, SPADE — "a super-acronym for single channel per carrier pulse code modulation multiple access demand assignment equipment". Even Mr Porter is moved to comment that "the jargon gets worse and worse but really there is no way to avoid it". Although agreeing with this, one wishes a glossary of acronyms had been included so that one does not have to hunt through pages to find the explanation of a particular term.

The section of the book dealing with the surveying of the oceans and the

land gives a good technical description of the "Earth's Resources Technology Satellites. Some of the illustrations used to demonstrate different spectral responses are disappointing, especially Fig. 7.6. Also one notes that the concept of the spectral signature, first mentioned on p98, does not receive further explanation until p127. Likewise, problems affecting the spatial extension of supervised and unsupervised classifications of digital data are not discussed. As a result, the casual reader may be left with the false impression that recognition of crop types is a simple matter.

The informed reader will feel that the present capability of LANDSAT in respect of mapping soils and land use is somewhat overstated. One may note that recent pronouncements by NASA officials are more cautious. For example, J. Morrison, (*NASA Earth Resources Survey Program: Problems and Prospects*, European Space Agency Symposium on Remote Sensing of Earth from Space, Strasbourg, 1977) writes "I do not think we will be able to do this (that is, the Worldwide Crop Information System) successfully until about the 1984 period. The reason is that the present LANDSAT satellites simply do not have the resolution necessary for providing worldwide crop data, particularly in small field situations". Likewise, the assertion that plant disease can often be detected in satellite images before it is apparent to the casual observer on the ground was not borne out by the Corn Blight Watch Experiment (R. B. MacDonald *et al.*, *Results of the 1971 Corn Blight Watch Experiment*, Proc. 8th Int. Symp. Remote Sensing of the Environ-

ment, 1, 157, Ann Arbor, Michigan; 1973). It seems likely that such achievements must await the launch of LANDSAT-D and the Thematic Mapper.

Perhaps the most interesting part of this book is the chapter entitled "Laboratory for Science; An Observatory for Astronomy". This presents the case for space experiments and space observations in a succinct and effective manner. Subsequent sections dealing with the manned satellites and the Shuttle programme are also useful. One feels, however, that the role of the European Space Agency in the Spacelab programme could have been given greater prominence. In contrast to the space race between the US and the Soviet Union, the Spacelab programme provides a welcome example of international collaboration. Further discussion of Spacelab would also have revealed some of the initial limitations of the Shuttle in respect of mission duration and maximum inclination of orbit when launched from the Eastern Test Range.

In the last paragraph, Mr Porter notes that the future depends on the use that is made of satellite technology. It is increasingly apparent that there are considerable problems of technology transfer to be overcome before satellite information will be used to its full potential. This book provides an interesting glimpse of the technologist's world and will help the potential user to understand some of the engineering constraints on satellite systems.

L. F. Curtis

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## Parasitic Protozoa

*Parasitic Protozoa.* Vol. 4: *Babesia, Theileria, Myxosporidia, Microsporidia, Bartonellaceae, Anaplasmataceae, Ehrlichia, and Pneumocystis.* Edited by J. P. Krier. Pp. xv+386 (Academic: New York and London, 1977.) \$32.50; £23.05.

I HAVE already commented on the fact that the scope of this series encompasses more than the Protozoa (*Nature*, 268, 773; 1977); and favourably, as the treatment of parasitic Protozoa along with other parasitic organisms of similar basic behaviour, multiplying in the vertebrate host, is more likely to be conducive to exchange of fruitful concepts than the traditional association of Protozoa with the helminths, organisms of a basically different mode of life. The present volume is an illustration of this useful format, presenting ten chapters dealing respectively with *Babesia* in domestic animals and in man and other hosts; with *Theileria*, *Myxosporidia*, *Microsporidia*,

*Bartonella* with *Grahamella*, *Anaplasma*, *Aegyptianella*; with *Eperythrozoon* and *Haemobartonella*, *Ehrlichia* and *Pneumocystis*. The volume deals with a range of organisms all of which are important in relation to disease in man or his domestic animals, interpreting the latter so as to include his cultivated fish and insects.

The reviews are excellent up-to-date summaries of their subjects, many of which are diffusely scattered in the literature. They will be popular as systematic first readings or as reference guides to the 'state of play' of their respective subjects. The book is well presented and indexed, with a few line diagrams and half-tones; the last do not receive special treatment as regards the paper on which they are printed and so are adequate rather than excellent.

W. H. R. Lumsden

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**nature**

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## Another nuclear option

THE world will need a new source of energy when the oil and gas runs out: on that most are agreed, from the Friends of the Earth to the toughest champions of the nuclear industry. But how much energy? By when? And from what source? These are highly political questions, but they involve a technical one: what sources of energy are there? This question in turn, however, is not entirely apolitical. The gigantic momentum of the nuclear industry, set up to tame the atom bomb and, no doubt, to salve the consciences of the physicists ("We have known sin," said Oppenheimer, after Hiroshima), has determined the principal option: the fission of uranium nuclei. There has been too little cash for options other than nuclear and too little work on them. One thinks first of such fashionable renewable resources as wind, wave, sun, and geothermal energy. But, it turns out, it is not only renewable resources that are under-researched. There are specifically nuclear options that have passed almost unnoticed—as our feature this week shows (page 376).

The nuclear industry cannot continue indefinitely with existing reactors, for they consume only a tiny part of natural uranium—the fissile isotope  $^{235}\text{U}$ . The bulk of the uranium, consisting of stable  $^{238}\text{U}$ , is "wasted". This was clear at the beginning of the nuclear power programme, so it was natural to develop a system which would burn the  $^{238}\text{U}$ . This was the fast breeder, which lets the chain reaction in the  $^{235}\text{U}$  go so fast that some of the released neutrons convert the  $^{238}\text{U}$  to fissile plutonium,  $^{239}\text{Pu}$ . The plutonium they "bred" can in turn chain-react to produce energy. This system is not only economical in uranium—it seems on the face of it to be necessary in a principally uranium-based economy, for the energy-equivalent reserves of  $^{235}\text{U}$  that are extractable with a net yield of energy amount to only one-fifth of the oil reserves. Hence the dash of the nuclear industry towards the fast breeder reactor.

Yet there is an alternative to the fast breeder which will still conserve uranium—the thorium-uranium cycle. Perhaps it was the military need for plutonium; perhaps it was the simple logic of reaching towards the untapped 99% of uranium, the teasing  $^{238}\text{U}$  nucleus, that concentrated all attention on the fast breeder. But the Th-U cycle, which, at least according to its growing band of advocates, avoids the tremendous power density of the fast breeder core ( $\frac{1}{2}$  MW per litre, compared with 3 to 100 kW per litre for a thermal reactor), which reduces the problem of the long-lived wastes like americium and curium, which protects against

illegal diversion of fuel (it becomes highly radioactive), and which with proton accelerators to "top-up" the fissile content of the fuel rods might even avoid reprocessing altogether, has been looked at hardly at all outside some work in Canada and the US.

Pressure to work on the cycle comes from those resisting the fast breeder, and also from an unexpected source—accelerator physicists. The latter are to be distinguished from the high energy physicists who merely use accelerators. In a time of declining budgets for high energy physics the accelerator physicists are looking for someone else to sell their accelerators to; and the energy business seems a good bet. Yet they should not be dismissed for their self-interest; they are ingenious people and their proposals should be looked at carefully. Already they have initiated what may be the fastest route to fusion power (ion beam fusion). In the Th-U cycle they are offering ways of improving the efficiency of the cycle beyond what seems possible in the Canadian CANDU reactors until the cycle reaches true breeding—the creation of more fissile nuclei, from a non-fissile source, than are consumed in the reactor. It seems impossible to consider the Th-U cycle without considering the contribution the accelerator physicists can make.

Once breeding has been established in the Th-U cycle the reserves of thorium, not uranium, are the ultimate constraint. There seems to be plenty of thorium about, with major reserves in Canada, India, and the USSR. There is probably quite enough extractable with sufficient energy economy to exceed the energy reserves of the fast breeder fuelled on uranium.

Leaving aside the vexed arguments about whether we need a nuclear power source anyway, whether it is even economic, and whether its capital intensity is appropriate for the Third World (which is potentially the world's major energy consumer), it would seem at least that the thorium-uranium cycle, with accelerator back-up, should be given very serious attention as part of the political energy equation. It may be, of course, that it is too late; that we will need our new sources of energy before Th-U can be developed into a commercial proposition. Or that the philosophically and—if you are of a certain leaning—politically attractive renewable resources, together with energy conservation and coal, will become practical and economic alternatives before you can say "fast breeder". In fact, given the opposition to the latter, Th-U plus accelerators may be the nuclear industry's last chance. □

# Conserving uranium without the fast breeder

The fast breeder, with all its dangers, is not the only way forward for the nuclear power programme. **John Davies** explains

NATURAL uranium, unaugmented by breeding, will provide a relatively small reserve of energy: uranium energy reserves amount to only one-fifth those of oil. The best-promoted nuclear solutions to this problem are the fast breeder reactor (FBR) and controlled fusion. The former is a means of stretching the uranium resources by two orders of magnitude; the latter promises virtually unlimited power from sea-water. Neither method is straightforward, although both were being discussed in undergraduate lectures twenty years ago. A commercial FBR is not yet operational and the design of a fusion reactor providing energy break-even is still in the unplanned future. Moreover FBRs, with their use of plutonium, are responsible for many of the strong, reasoned objections to nuclear power. However, few have realised that there are several other ways of breeding fissile materials that avoid fast reactors and their problems.

Breeding—creating fissile from non-fissile material—requires an intense source of neutrons. Neutrons can be produced by an FBR; or by a fusion reactor (yet to be built); or by using proton accelerators. The last option has not been widely discussed.

To make the arguments clear, I shall first describe some of the basic physics occurring in reactors, particularly to explain what "breeding" is and why it is essential. I shall then look at the objections to FBRs before examining the alternative means of breeding to demonstrate a system that I believe is practical, economically viable, and safe.

As medium-weight nuclei are the most stable, considerable amounts of energy are generated either when two light nuclei fuse together, or when a heavy nucleus splits into two smaller ones (undergoes fission). To exploit either possibility requires an energy barrier to be overcome. (If there were no energy barrier all light or heavy nuclei would by now have fused or fissioned leaving only medium-weight nuclei.) Another requirement (in fission) is a mechanism for maintaining a chain reaction—where the fission of one nucleus in turn induces the fission of others.

In fission, the energy barrier is, roughly speaking, the surface tension of the nuclear droplet. A heavy nucleus approaching fission becomes progress-

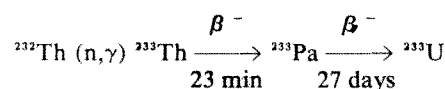
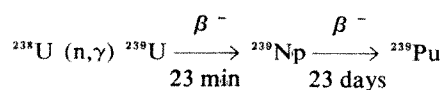
ively distorted, and the energy of the increased surface provides the fission barrier (small droplets of water are stable for the same reason). The impact and interaction of a neutron of the right energy overcomes this energy barrier. If, in turn, each resulting fission releases more neutrons (as it will, as heavy nuclei are neutron-rich) the neutrons will maintain a chain reaction with an unending succession of nuclei being induced to split.

Let us now consider fission in more detail. Thermal reactors use the fissile nuclei  $^{235}\text{U}$ ,  $^{239}\text{U}$  and  $^{239}\text{Pu}$  where the binding energy provided by the capture of a thermal neutron is sufficient to overcome the surface energy barrier against prompt fission of the resulting  $^{234}\text{U}$ ,  $^{236}\text{U}$  and  $^{240}\text{Pu}$ . Each induced fission also provides several neutrons which can then start a chain reaction. But the trouble is that the isotopes  $^{235}\text{U}$  and  $^{239}\text{Pu}$  are artificial; and  $^{238}\text{U}$  is a rare isotope of uranium, the most abundant being  $^{238}\text{U}$ .

Unfortunately, the abundant  $^{238}\text{U}$  will not do for fission, as the figure shows. This plots the effective number of neutrons per fission ( $\eta$ ) against the kinetic energy of the captured neutron; and shows the fission neutron energy spectra in thermal and fast reactors. In contrast with (say)  $^{235}\text{U}$ , the last, odd neutron in  $^{238}\text{U}$  has a smaller binding energy. Consequently only the most energetic neutron coming from fission can cause further fission by capture in  $^{238}\text{U}$  and so  $^{238}\text{U}$  cannot maintain a chain reaction.

With thermal reactors only, using natural uranium, uranium resources are insufficient to maintain a long-term nuclear energy programme. The only naturally occurring fissile isotope is  $^{235}\text{U}$ , present at 1 part in 140 of  $^{238}\text{U}$ . Moreover it is only part-burnt in thermal reactors. This leads to the idea of trying to "breed" fissile isotopes, by using the excess neutrons in the chain reaction to create fissile nuclei in otherwise non-fissile elements.

From each fission of  $^{239}\text{U}$  one neutron is required to maintain the chain reaction and 0.2 neutrons escape. Any excess over 1.2 neutrons could be used to convert  $^{238}\text{U}$  or  $^{232}\text{Th}$  to fissile  $^{239}\text{Pu}$  or  $^{233}\text{U}$  via



(Here the notation  $A(n, \gamma)B$  means nucleus A absorbs an incoming neutron and becomes nucleus B, emitting a gamma ray (a photon) in the process.

The notation  $B \xrightarrow{\beta^-} C$  means B decays by  $\beta^-$  emission to C with a half-life of  $x$  minutes.)

The difference between the two reactions is that the first needs fast neutrons, while the second can use slow ones. The reaction  $^{238}\text{U} \rightarrow ^{239}\text{Pu}$  occurs to a small extent in present thermal reactors (in so far as there are a few fast neutrons around) and chemical processing can enable up to 1% of all available uranium to be burnt. Even so there is probably little point in planning further thermal reactors (as there will be insufficient uranium to fuel them and existing reactors).

## Breeding in a fertile blanket

The fissile material for reactor fuel can be produced from fertile material using the excess neutrons from a reactor and the above reactions. In practice this could be done by surrounding the reactor with a 'blanket' of fertile material where the spare neutron would then 'breed' new fuel. In fact if  $\eta \geq 2.2$ , with one more neutron per fission than required to maintain the chain reaction, then breeding in a fertile blanket will maintain or increase the fissile content and allow almost all the uranium to be burnt. The figure shows that a fast reactor burning  $^{239}\text{Pu}$  surrounded by a fertile  $^{238}\text{U}$  blanket absorbing the excess fast neutrons is the best bet.

Is this really true? Much of the reasoned objections to nuclear power are consequent on the following problems of the fast breeder reactor:

- New technology—breeding around a 500 MW dustbin whose reactivity—burning rate—can be accidentally increased in micro-seconds; no artificial control is fast enough.

- Time—whether thermal reactors can produce enough  $^{239}\text{Pu}$  to fire a FBR regime whose time to breed the initial fissile content is anyway commensurate with their working life.

- The expensive, messy and dangerous chemical separation of fissile materials, in particular  $^{239}\text{Pu}$ , from spent fuel elements. This separation greatly increases the possibility of

- $^{239}\text{Pu}$  being diverted into nuclear weapons; this is further exacerbated by the considerable transporting of fissile material and by inaccuracies of assay of  $^{239}\text{Pu}$ , which can be so large as to be greater than the amounts needed for

many bombs. (This is called the "Muf"—materials unaccounted for—problem.)

● The cost—as yet not evaluated—capital, running and political—especially for trying to make them safe.

Thermal reactors on the other hand involve established technology. The figure shows that such a reactor fuelled with  $^{233}\text{U}$  has a breeding gain ( $\eta-2.2$ ) greater than 1.00 i.e., as much  $^{233}\text{U}$  will be created in a  $^{232}\text{Th}$  blanket (absorbing the slow neutrons) as is burnt as fuel. Unlike  $^{239}\text{Pu}$ ,  $^{233}\text{U}$  is automatically safeguarded against illegal diversion into bomb-making by the intense  $\gamma$ -rays coming from the simultaneously produced  $^{232}\text{U}$ ; additionally one can add sufficient  $^{238}\text{U}$  to make life difficult for the bomb maker but still keeping the  $^{233}\text{U}$  fissile. Such reactors avoid plutonium and the technological problems of FBRs. Also they ease the waste problem: the proportion of difficult transuranic elements (which take a long time to decay) is considerably reduced.

Thermal reactors fuelled on  $^{233}\text{U}$  can have a breeding gain of 1.00 or greater in a Th blanket. But considerable development would be necessary to make this economic. Additionally chemical re-processing of the spent fuel rods, as yet undeveloped, may be necessary.

Consider what a well established, practical and economic reactor can do with a Th-U cycle. The Canadian Deuterium Uranium (CANDU) series of heavy water moderated thermal reactors have high neutron economy and use natural uranium so there is no chemical separation. They can operate on a Th-U cycle with breeding gain as large as 0.92. Thus to use all the available thorium requires only 8% of the  $^{232}\text{Th}$  atoms to be converted to  $^{233}\text{U}$  by non-nuclear means.

Some fusion enthusiasts are lowering their expensive sights, ignoring energy output and promoting fusion reactors as intense neutron sources for fertile to fission conversion. This is not surprising! Fusion reactor design is replete with problems such that only a 2000 MW TOKAMAK has promise of creating as much energy as it consumes even with the most easily ignited  $\text{D}(\text{T}, \text{n})$   $^4\text{He}$  reaction. It is possible that the 14 MeV neutron is far more valuable for breeding than for heating, each neutron converting two or more fertile atoms into fissile ones. Even so despite the relaxed conditions for only providing neutrons fusion technology is still a dream and many regard a hybrid arrangement as combining the worst features of fission and fusion.

### Proton accelerators

Of all the big advanced technologies, that of accelerator building can claim to have established the most consistent

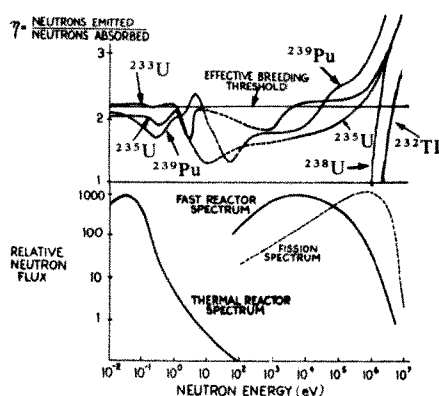
track record. The high intensity accelerator at Los Alamos is well on the way to its design beam current, the CERN SPS is working ahead of schedule within its design budget while the high energy accelerator at the Fermi Laboratory is already  $2\frac{1}{2}$  times its original design energy. At several laboratories high intensity accelerators are now replacing fission reactors as research instruments providing high intensity neutrons.

The intense neutron fluxes from such particle accelerators can effect fertile-to-fissile conversion and so produce far more energy than they consume. A 1 GeV proton interacting in a uranium target produces 60 low energy neutrons by a process known as spallation; each neutron can change one  $^{232}\text{Th}$  atom into one  $^{233}\text{U}$  atom in a fertile thorium blanket surrounding the uranium target; each  $^{233}\text{U}$  atom can fission in a CANDU reactor producing 200 MeV of available kinetic energy; of every 100  $^{233}\text{U}$  atoms so burnt, 92 will have been produced in a thorium blanket around the same or similar reactor while only 8 will have had to be produced via an accelerator. So effectively a single 1000 MeV proton produces

$$100 \times \frac{60}{8} \times 200 = 150,000 \text{ MeV}$$

This is possibly a lower estimate as there may be neutron multiplication in the blanket.

Accelerators are inherently cheaper to build than reactors and a large proportion of the accelerator operating power could be provided from heat generated in the target. The accelerator design criteria are high efficiency of radio-frequency power to beam energy conversion, minimal power usage in other parts of the accelerator (such as magnets) and high extraction efficiency.



The ratio of neutrons emitted to neutrons absorbed, in collisions between neutrons and nuclei, plotted against neutron energy. Also shown are the neutron spectra from fission directly (dotted line) and in a thermal and fast breeder reactor

Most work on meeting these requirements has involved a high intensity, medium energy, linear accelerator which is essentially an extension of the 800 MeV Los Alamos machine, now being worked up to 1 mA beam current. However, as a good example of lateral thinking, is a suggestion that these requirements can also be met by a low current, very high energy accelerator, an extension of the 400 GeV CERN SPS or the 500 GeV accelerator at the Fermilab.

A Canadian group including Fraser, Hoffman and Tunnicliffe at Chalk River are designing a 100–300 mA, 1 GeV proton linear accelerator that would breed enough fuel to supply the 8% deficiencies of a dozen, 1,000 MW CANDU reactors operating on a uranium–thorium cycle. The fertile blanket would consist of new fuel pins that would subsequently go directly to the reactor after a pause to allow for  $^{233}\text{Pa}$  decay. A sophisticated fuel management scheme is conceivable in which partially-burned fuel pins are upgraded by re-insertion in the target and then returned to the reactor.

Another suggestion, from Wilson at Fermilab, is for a superconducting 1000 GeV accelerator with an intensity of  $1-2 \times 10^{13}$  protons per second. At this energy the number of spallation neutrons is still increasing linearly with energy and so a single proton will produce 60,000 neutrons in a block of uranium. High energies and low currents mean low beam loss and efficient conversion of radio-frequency power to beam energy for protons travelling close to the velocity of light. Fermilab's superconducting energy doubler will provide 1,000 GeV protons. The intensity required for energy break-even must be increased from the present  $10^{12}$  protons per pulse by an amount that one has come to expect over an accelerator's life-time. This

neglects the effective  $\times \frac{100}{8}$  energy

multiplication obtained by breeding  $^{233}\text{U}$  for CANDU reactors.

Accelerator spallation neutron sources are being built at several laboratories as research instruments to replace fission reactors as a source of high intensity neutrons. Accelerator scientists regard upgrading spallation sources for breeding purposes as a reasonable extrapolation of existing, successful techniques. This would make the thorium–uranium cycle in thermal reactors a serious contender as a nuclear fuel source with the added bonus (to many the over-riding attraction) that objectionable material need never be removed from the fuel rods. □



## The Ganges shared out

BANGLADESH and India have agreed to share the water from the Ganges and to increase the river's flow. The interim agreement lasting five years, which the two countries signed on 5 November, states that they will share the water during the dry months from 1 January to 31 May. Estimates of the amount of water available are based on the recorded flow of the Ganges at Farakka from 1948 to 1973. The water will be released to Bangladesh for ten days at a time in three instalments each month from January to May.

The agreement also provides for water sharing during the driest period from 21 April to 30 April. At that time, water sharing between India and Bangladesh will be at the rate of 20,500 cubic feet per second and 34,500 cubic feet per second respectively. The total flow at Farakka during the period averages 55,000 cubic feet per second. The actual availability above or below the amount calculated for the period will be shared between the two countries in the same proportion. The signing of the agreement marks the culmination of protracted negotiation between the two countries which has lasted for 25 years and it is thought to be unique between riparian states.

The agreement was signed at about the same time as the Second Committee of the United Nations Environmental Programme (UNEP) constituted an intergovernmental group of experts to draft guidelines for sharing natural resources harmoniously between states. A Bangladesh representative has proposed that a conference on international rivers be organised to evolve and strengthen an institutional framework on a national, regional and international basis to deal with the problems of exploiting and developing international rivers. It is



also suggested that UNEP should be responsible for advising on the development and non-wasteful use of national resources based on sound environmental policy.

The importance of the Ganges to Bangladesh can be judged from the fact that the Ganges delta covers about 37% of the total area of Bangladesh, and contains about one-third of the total population of the country. The river provides water for drinking and other domestic and industrial uses, sustains agriculture, forestry and fisheries, and facilitates in-land navigation. The increasing demand for greater agricultural output means that water is needed throughout the year. The water available during the dry months may not be enough for the four thousand acres of paddy land dependent on the Ganges.

The task of feeding the fast growing

population of Bangladesh from the limited land and water resources is a challenging problem. Cultivating rice, the staple food of the people, is confronted with two serious handicaps—the excess of water during the monsoon and its scarcity throughout the dry months. Controlled irrigation is an alternative but expensive proposition. The Bangladesh Planning Commission, in consultation with the Water Resources Division and the Water Development Board, has proposed four pilot projects, for four different zones of the country, to develop a way of conserving water during the wet season and making it available to the farmers for the rest of the year, particularly during the lean months. The water conservancy programme will also provide a suitable environment for pisciculture and other aquatic sources of food.

**M. Kabir**

## UK Energy Commission meets

THE DESIRE of Mr Tony Benn, Britain's Secretary of State for Energy, to see a fully-integrated national energy policy came a step nearer to fruition on Monday with the first meeting in London of the newly-established Energy Commission.

The commission is an advisory body set up earlier this year to provide a regular forum for representatives of both the producers and consumers of energy, to discuss long-term strategy and specific problems.

Mr Benn, speaking after Monday's meeting, said that he was aware that the Energy Commission did not represent as powerful a set of voices on

alternative as on traditional sources of energy—at least as far as research and development was concerned.

"The Government has been criticised by Sir Brian Flowers, as chairman of the Royal Commission on Environmental Pollution, and others for not paying enough attention to alternative sources of energy, and this is a criticism which we accept," he said.

One of the first requests which he had made to Sir Hermann Bondi, recently appointed chief scientist at the Department of Energy, was to look at the balance of research effort and investment between conventional and more novel forms of energy produc-

tion.

A great advantage of research in this area was that it was relatively cheap, at least in comparison with the capital intensive investment stages. "It would be sensible for us to expand our support for research over a wide range of possibilities, and then let the development stage bid for part of the energy budget. This is an area in which I am convinced that, given the determination, we can make more efforts more quickly."

The 24-members of the commission include representatives of the energy-producing industries, relevant trade unions, various employers' organisations and consumer bodies. It has been created by Mr Benn following last year's National Energy Conference, at

which the issue of energy policy was for the first time in Britain officially pushed into the arena of public debate.

According to its terms of reference, which were announced by Mr Benn in July, the commission will be concerned with two main issues: the developments of a strategy for the energy sector in the United Kingdom, and specific aspects of energy policy that arise from time to time.

On both of these, according to Mr Benn, the commission will "seek to form an agreed view", feeding into the process by which he hopes that the Government will eventually be able to publish annual reports on the energy situation. These would contain reviews of decisions taken, current prospects, and matters likely to come up for discussion.

As a first step, the Government is expected to publish a Green Paper on energy policy in Britain early in the new year. This will be based on a working document on energy policy which was prepared for the Energy Commission, and published by the Department of Energy last month.

The working document, which was welcomed by environmentalists as indicating slightly more tentative attitude

towards energy issues than previous government statements, and reduced by 10 per cent an earlier forecast of energy needs in the UK by the year 2,000, was one of the main items on the agenda of Monday's meeting.

Among the comments made, for example, was that little attention had been given to the problem of the availability of skilled manpower for the energy industries, a point which Mr Benn said would, like others made at the meeting, be taken into account in the drafting of the Green Paper.

Mr Benn expressed satisfaction, however, that in line with what he calls a "consensus approach to energy policy", the members of the commission had given general support for the working document which was to form the basis of the Green Paper. "We have hit on an energy programme for December 1977 which can claim a wide measure of public consent and understanding", he said.

He emphasised, however, that the commission was purely advisory. Although it provided a chance for the various power industries to compare notes on their future programmes, there was no suggestion that the commission should take over either the

responsibilities of the fuel industries, or those of the established Parliamentary and Governmental channels.

It remains to be seen whether this will be sufficient to maintain the confidence both of the nationalised industries, with their recently-expressed concerns about government "interference", or the trade union representatives, who would like a body such as the commission to assume a great executive responsibility.

Earlier this year Mr Frank Chapple, general secretary of the Electrical, Electronic, Telecommunication and Plumbing Union, and chairman of the TUC's Fuel and Power Industries Committee, six of whose members represent the trade union interest on the commission, suggested that there was a need for a body such as the commission to "bang heads together and work out mutually agreed policies".

Mr Chapple warned: "We are not prepared to rescue the formulation of energy policy from the short-term vagaries of the market-place only to see it taken over by bureaucrats in Whitehall and removed from the public domain."

David Dickson

## A second bang in the Urals

THE Kyshtym event of 1958 in many ways parallels the Tungus event of 1908. Both took place in darkest Russia. Both attracted virtually no outside attention for almost two decades. And both, once publicised, became the subject of much speculation, both informed and uninformed, with every new piece of evidence only adding to the mystery.

One of the few clear pieces of evidence to what happened at Kyshtym is given by Dr Lev Tumerman, now of the Weizmann Institute. He visited the area in 1961 and reports extensive devastation, typical of a nuclear disaster. Dr Tumerman, however, will not commit himself on the cause, stating only that it could not have been an accident in an operational power station, since at that time the Soviet Union possessed no such installation. Even in 1961, the first such station, at Beloyarsk, was still in the foundation-laying and concrete mixing stage.

Dr Zhores Medvedev, who visited the area at much the same time, is convinced that the explosion occurred in a nuclear waste store. Although the storage of waste products involves a real leakage hazard, it is difficult to see how it could cause an explosion. One Russian scientist (not a nuclear physicist), told *Nature* that he himself felt that Medvedev's explanation was quite possible, since the Soviet regime pro-

duces in its scientists an "attitude of neglect", so that proper precautions are everywhere ignored. This explanation would, however, apply equally to an explosion at a prototype reactor plant, as much as at a waste dump.

The latest step in the enigma is posed by the release of hitherto secret documents by the CIA. Fourteen documents were released; another 15 exist but are still considered too sensitive, so that, as seems typical for Kyshtym, only partial evidence is available. The involvement of the CIA itself raises a number of questions; in particular, was the notorious U-2 flight over the area

simply part of a routine Union-wide patrol, or was the unfortunate Gary Powers sent to confirm reports already received from a ground-level contact?

Much more mysterious is the fact that the CIA material mentions *two* explosions, one in 1958 and one in 1959. It seems on the face of it unlikely that two nuclear accidents could occur in successive years—even if we admit that an atmosphere of neglect makes Soviet installations peculiarly accident-prone, the fall-out from the first disaster would have surely led to the abandonment of the area, with the shut-down of any project already operating.

Vera Rich

## Was Tungus an astronaut?

SINCE the first expedition, just 50 years ago, to the site of the "Tungus event" of 1908, the explosion has posed a major riddle to geophysicists. The latest in a long line of theories, that of a certain Aleksei Zolotov, poses a further mystery—how, in the conditions prevailing in Soviet science, did it ever get published?

It is well-known that the publication of scientific articles in the Soviet Union is subject to strict controls. No paper may be submitted without a special certificate from the author's place of employment—a regulation which, it would appear, leaves no outlet for either the gifted amateur or the harm-

less crank. When Soviet science has, on occasion, followed its own course, unacknowledged by the outside world, this has been due to either the party bypassing normal academic procedures—as in the case of Lysenko—or else to the initiative of the topmost echelons of the scientific establishment—as with the blanket denial of ocean-floor spreading, or Snezhnevskii's theory of "creeping schizophrenia".

Recently, however, Moscow radio broadcast in both Russian and English a "new explanation" of the Tungus "enigma". The theory itself is not new to Western readers of fringe publications: the devastation, it is claimed,



was due to the explosion of an artificial "alien body", and involved nuclear reactions. Within the apparently monolithic structure of Soviet scientific publishing, its appearance, however, is startling.

There have been a number of expeditions to the Tungus area in recent years. Detailed analyses of soil and peat samples have been made, revealing, *inter alia*, a large quantity of silicate particles (diameter less than 200  $\mu\text{m}$ ) in the 1908 peat layers near the epicentre. Last March, Professor Emlen V. Subbotovich, the geophysicist, announced the findings of these expeditions: all previous theories, he said, including anti-matter, meteorite, or alien spacecraft, were wrong. The explosion was caused by a comet-like body, with density not greater than 0.9  $\text{g cm}^{-3}$ . His pronouncement was made with all the concomitants of a definitive pronouncement. Where, then

does Zolotov's theory fit in?

Last week, a former member of several Tungus expeditions visited London—Dr Khronid Lyubarskii, who was recently exiled for his part in the dissident human-rights campaign. Dr Lyubarskii, an astrophysicist specialising in the physics of meteors and meteorites, fully endorsed the official view that a comet was responsible for the Tungus event. According to him, Zolotov, who was described in the broadcast as a mathematician and physicist, was originally an engineer from an oil prospecting team. How he first became involved with Tungus is not clear; the impression which Dr Lyubarskii gave of him is, however, a familiar one—the self-appointed "expert" who in his own opinion knows far more than the scientist in the field. Zolotov's article, it turns out, did not appear in any scientific journal; hence no "certificate" was needed. It was

published in the monthly *Turist*—perhaps in the hope of attracting holiday-makers into the further reaches of Siberia. And for popular journals, explained Dr Lyubarskii, while there is strict political censorship, there is no control of "scientific" (or pseudo-scientific) content.

It is perhaps inevitable that genuine scientific research is surrounded by a penumbra of more or less wild speculation; indeed, this may be a necessary factor in the growth of science. One of the dangers of a monolithic state-controlled scientific establishment is that the rigid structure of the system can exclude the brilliant amateur, and also the scientist trained in one discipline, who, like Pasteur, does his most significant work in another. However wild Zolotov's theory, the fact that it can still find an official outlet in the Soviet Union is a happy omen.

Vera Rich

FOR years, many adult middle-class males have tried to substitute diet for exercise in warding off heart attacks. The research effort on this "diet-and-heart" question, says George Mann, "has ended in disarray." Dr Mann's conclusions were noted in *Nature* (3 November, page 2) by Dr Rivers.

The popular dogma, which dates from around 1950, has said that diets low in saturated fats and cholesterol, but containing polyunsaturated fats (PUFAs), will produce lower blood-cholesterol levels and will lessen the risk of coronary heart disease. The idea was well-accepted; it was supported by many prominent clinicians and nutritionists. Sales of vegetable oils grew apace, especially those oils for which a slightly higher-than-average content of esters of essential PUFAs could be claimed. Big fields of thistle-like safflowers yellowed the landscape with their blossoms. Safflower seeds furnished the *cordon bleu* of vegetable oils, so rich in PUFAs that you could just feel it doing you good. The oil was hydrogenated to make margarine, even though, as George Mann points out, the hydrogenated, and hence trans, fatty acids have a hypercholesteremic effect. Butter, cream and eggs were to be shunned.

What was the effect of 25 years of these measures? By 1977, as Dr Mann points out, the results show that "a huge cohort of persons past middle age has been recruited to a futile regimen of dietary restrictions". No diet therapy has been shown effective for the prevention or treatment of coronary heart disease. Two of five dietary regimes in which vegetable

oils were featured showed an increased incidence of deaths from cancer in the treated groups.

This may be meaningless, but several experiments have shown an increase in tumour incidence in rats on diets high in polyunsaturated fats,

## Sursum corda



THOMAS H. JUKES

such as sunflower-seed oil and corn oil, as compared with saturated fats.

The vegetable oil motif was for many years the official guide to cooking and eating. Who would dare to challenge the sonorous pronouncements of medical authorities when the punishment for such heresy was incapacitation or death? We could almost feel the waxy cholesterol and tallowy fats clinging to our arterial walls after we suicidally wolfed down scrambled eggs or lamb chops. Thoughtful, health-oriented advertise-

ments gave us tasty recipes, made with vegetable oils. Sugar was added to the list of culprits; it could allegedly give rise to "hard fats" when metabolised. In any case, sugar is sweet, and therefore, sinful.

A new ascetism burgeoned and, true to type, its main objective is restoring sybaritic evil-doers to the path of dietary righteousness. Its leader is Senator George McGovern, whose committee on 'Nutrition and human needs' has issued a preposterous report on 'Dietary goals' which calls for governmental action to implement the prejudices of its writers.

The report says, among other things, that it is necessary [*sic*] to make partial substitution of polyunsaturated fat for saturated fat. Why? What about the rats with cancer? The report quotes the *New York Times* to support its thesis for reducing sugar consumption by about 40%, and speaks ominously of cola-guzzling youngsters (the little devils!) and the need for protection against "hidden sugar". Martini-guzzling adults get off without reprimand or umbrage, but we are told that monosodium glutamate "may be associated with headaches, flushes in the head and body and tingling in the spine". Since most common vegetable food proteins contain 20% or more of glutamate, there should be much tingling after a meal, and don't blame the martinis.

Avast to McGovern and his anonymous bluenoses! 'Tis the season to be jolly! I don't think they know what they're talking about, anyway, even if they have wangled a multi-million dollar appropriation to brainwash the public.



# correspondence

## In support of catastrophe theory

On 27 October we published an article by R. S. Zahler and H. J. Sussmann (page 759) critical of 'incorrect reasoning, far-fetched assumptions, erroneous consequences and exaggerated claims' in biological and social-science applications of catastrophe theory. Here is a selection of responses to the article

SIR,—Zahler and Sussmann have a basic misunderstanding of catastrophe theory: in their criticism they ignore the fundamental concept of stability. Stability lies at the root of the modern mathematical theories of dynamical systems and singularities, of which catastrophe theory is a part. The concept was introduced by Andronov and Pontryagin in 1937, and it has been greatly developed not only by Thom but also by the Russian school, notably Anosov and Arnol'd, and the American school, notably Whitney, Smale and Mather.

The importance of stability in modelling lies in the fact that if a stable model is perturbed, then its qualitative properties are preserved. Thom acknowledges the mathematical and scientific importance of stability by incorporating it into the title of his book *Structural stability and morphogenesis*, in which he first introduced catastrophe theory. On page 762 of their article Zahler and Sussmann ask the rhetorical question: "So we must ask again: what is special about a model that looks like a cusp?" and the simple answer to their question is that the cusp catastrophe is stable, whereas their figure 4 is not.

For details of their criticism Zahler and Sussmann refer the reader to a longer paper of theirs, which is not yet published. However, in the preprint of this paper, which they have circulated widely, there are major mathematical mistakes underlying their main criticisms: I explained some of these mistakes at length to Sussmann when he visited Warwick University in July this year.

Most of Zahler and Sussmann's scientific criticisms are based on misquotations, misunderstandings, misrepresentations, or quotations out of context. I give a typical example. On page 762 under the heading of 'Careless discussion of evidence' they state a number of so-called "facts", including: "Zeeman's embryology paper (*Lectures on Maths in the Life Sciences* 7, 69 (1974)),

besides being mathematically wrong, betrays the author's inexperience in embryology. For example (p. 27), Zeeman likens the embryonic neural tube to a roll of stiff paper which tries to maintain its curl. But experiment shows that cut neural tube persistently tries to unroll".

In these two sentences Zahler and Sussmann manage to misquote both Crelin and myself. Far from contradicting the metaphor, Crelin's experimental work supports it: Crelin writes (*J. exp. Zool.* **120**, 561 (1952)). "The grafts of all the embryos in the rotation series showed a tendency to curl in a matter of seconds after they were severed from the brain. Therefore, if the rotation of the tectum were delayed for some reason such as the graft sticking to the forceps, the graft would curl into the shape of a ball, making it impossible to continue the operation". On page 577 figure 19, Crelin shows a photograph of the graft curled into a ball. Meanwhile on page 127 of my paper (there is no page 27) it is the underlying mesoderm, not the neural tube, that I liken to a roll of stiff paper. The burden of my discussion on that and the preceding pages concerns the forces exerted, *in vivo*, by the underlying mesoderm upon the overlying ectoderm and neural plate, before the latter has rolled up into neural tube, and at a much earlier embryonic stage than Crelin's experiment.

I now give an example of misrepresentation. When sophisticated mathematics is applied to science it is common practice to publish separately both rigorous mathematical proofs and more simplified expositions of the same material: the latter are essential if the work is to be made available to those scientists who are not expert mathematicians. A case in point is my treatment of primary and secondary waves in developmental biology. My initial simplified version, addressed primarily to biologists, is in *Lectures on Maths in the Life Sciences*, while mathematical discussions and rigorous proofs, addressed primarily to mathematicians, are in *Proc. Int. Cong. Math., Vancouver* **2**, 533 (1974) and Wasserman, G. *Acta Math.* **135**, 57 (1975). The mathematical treatment uses stability with respect to a symmetry-group that is appropriate to developmental biology, namely an extension of the group of diffeomorphisms of space-time preserving the foliation by time-paths. Zahler

and Sussmann criticise the simplified version for mathematical naïvety, without acknowledging the existence of the sophisticated version, although the latter has been brought to their attention long ago.

These are two examples: I could point to a hundred others. I have always found that my work has benefited from the constructive criticism of my fellow mathematicians and scientists. However, to argue in print against the determined misrepresentations of Zahler and Sussmann is both tedious for the reader and unproductive, and an adequate answer to this type of criticism is provided by my original papers *Catastrophe theory, Selected papers 1972–1977*. Nevertheless I am prompted to reply to this particular article for three reasons: firstly, their disgraceful omission of any reference to the work of Thom on biology, secondly, their implied dismissal of the fine work of Berry in physics, and thirdly, the potential harm that their article might cause to the work and the careers of other scientists who are using catastrophe theory, particularly the younger ones who have not yet established their reputations.

E. C. ZEEMAN

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SIR,—In response to the criticisms of R. S. Zahler and H. J. Sussmann we wish to point out that contrary to misleading statements they make such as "catastrophe theory is a blind alley", several examples of genuine applications of catastrophe theory already exist in the physical sciences.

For example one of us recently studied the topological behaviour of stagnation points in two dimensional flows where, with the aid of Thom's elementary classification of degenerate critical points, a physical understanding was obtained of the complex behaviour of degenerate and non-degenerate stagnation (critical) points in a particular flow (Berry, M., & Mackley, M. R. *Phil. Trans. Roy. Soc.* **287**, 1–16 (1977)). Further physical applications amenable to direct experimental test exist, some of which were discussed at the Institute of Mathematics and its Applications meeting held at University College London in May 1977.

Concerning the application of catastrophe theory to biology, we agree that some inaccurate or premature claims have been made. In particular, Zeeman's argument for the existence



of primary and secondary waves in developing embryos does not have the logical status of a proved theorem since he has not as yet published the full mathematical proof. Rather, his use of catastrophe theory in a description of differentiation gives rise to the hypothesis that such waves occur and, with the additional postulate of a temporal periodicity of state in the tissue, this application suggests how spatially periodic structures such as somites may arise.

These hypotheses have stimulated experimental investigation, which is a major purpose of model-building. Furthermore, Zeeman's treatment of differentiation has the additional virtue of providing a unitary field description of a process which is often erroneously and misleadingly described in terms of separate spatial and temporal mechanisms. In a subject such as developmental biology, which has barely begun to come to grips with its central problem of morphogenesis in terms of models, it is more important to get the correct qualitative treatment than to attempt quantitative precision.

It is far too early to decide whether or not catastrophe theory will be of major value in biology. That it provides useful and accurate descriptions of certain physical processes is now beyond question. More generally, the context for catastrophe theory is topology, and topological thinking has been of immense value in the understanding of many physical phenomena. It seems highly probable that the topological approach will prove invaluable in the study of biological processes as well, but this is an approach that can only be learned slowly, with trial and error. Zahler and Sussman have presented some valid criticisms of applied catastrophe theory, but their over-reaction is unfortunate. It leads them into exaggeration and wholesale rejection of very useful propositions.

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B. GOODWIN

M. R. MACKLEY

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SIR,—The case in favour of catastrophe theory rests not on speculative models in the social sciences, but on successful applications to the physical sciences. In 1975 and 1976 there appeared approximately 42 papers applying catastrophe theory to physics, nine to biology, and 14 others: Sussmann and Zahler's criticisms deal almost entirely with one sociological paper, two on biology, and one model taken from two popular articles and a paragraph in a conference report. They do not hesitate to extend their conclusions to areas they have not studied: "we anticipate that

the results of an extended search (covering biology, linguistics, physics, or psychology) will be similar (that is negative)" from (Sussmann, H. J. & Zahler, R. S. *Proceedings of the 1976 biennial meeting of the Philosophy of Science Association, Chicago*, in press). Tim Poston and I have written a book (Poston, T. & Stewart, I. N. *Catastrophe theory and its applications*, Pitman, London, 477 pp.), due in print early in 1978, documenting quantitative applications in the sciences, which casts severe doubt on their conclusions. A major plank in their case—allegation of a repeated mathematical error—is refuted by Poston (*Mathematics Report*, Battelle Geneva (in press)). Their reliability may be judged by their statement: "Stewart repeats the untrue assertion that Zeeman's embryological predictions have been 'recently verified by experiment' ". What I wrote was: "... with the prediction that slowing down the chemical reactions of the primary wave would lead to the formation of fewer somites, an effect recently verified by experiment". Which happens to be true.

Similar misinterpretations vitiate many of Sussmann and Zahler's criticisms, rendering them analogous to disproving Pythagoras' theorem by exhibiting a triangle that is not right-angled. With the exception of their discussion of the nerve impulse model, few of their criticisms are conclusive, and some are simply wrong. Others are problems of general mathematical modelling, which can usually be resolved by reference to current scientific practice. Sussmann and Zahler's charges go considerably beyond anything they have correctly substantiated.

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SIR,—It would be a pity if the strong attack by Zahler and Sussman on some biological and sociological models based on catastrophe theory, (27 October, page 759) were to mislead readers into thinking that such new and beautiful mathematics has no useful application in any science. The fact is that in this laboratory catastrophe theory is being employed in the development of new concepts, in the explanation and prediction of phenomena, and in the design of experiments, in two areas of physics.

The first is short wave optics (and quantum mechanics) where Thom's theory classifies the forms of focal surfaces (caustics) and makes it possible to give a precise description of the finest detail in the associated diffraction patterns (Arnol'd, V. I. 'Critical points of smooth functions and their normal forms' *Uspekhi Mat Nauk*

(translation: *Russian Mathematical Surveys*) **30**, 1–75 (1975); Berry, M. V. 'Waves and Thom's Theorem' *Adv. in Phys.* **25**, 1–26 (1976); Duistermaat, J. J. 'Oscillatory integrals, Lagrange immersions and unfolding singularities' *Comm Pure App Math* **27**, 207–281 (1974)). The classification describes caustics that are 'structurally stable', that is those whose forms survive perturbation. This makes catastrophe theory particularly suited to the optics of nature rather than artefacts such as microscopes and telescopes whose focussing is dominated by cylindrical symmetry.

We have made progress in understanding the optics of irregular water droplet 'lenses' (Berry, M. V. 'Waves and Thom's Theorem' *Adv. in Phys.* **25**, 1–26 (1976); Nye, J. F. 'Optical caustics in the near field from liquid drops' (submitted to *Proc. Roy. Soc.*), the fine structure of swimming pool caustics (Berry, M. V. & Nye, J. F. 'Fine structure in caustic junctions' *Nature* **267**, 34–6 (1976)), atom scattering by crystal surfaces (Berry, M. V. 'Cusped rainbows and incoherence effects in the rippling-mirror model for particles scattering from surfaces'. *J. Phys. A* **8**, 566–84 (1975)) and the statistics of twinkling starlight (Berry, M. V. 'Focusing and twinkling: critical exponents from catastrophes in non-Gaussian random short waves' (*J. Phys. A*, in press)). This last application (which has proved peculiarly resistant to more conventional forms of analysis) makes essential use of the enormous extension of Thom's classification being developed by Arnol'd (Arnol'd, V. I. 'Critical points of smooth functions and their normal forms' *Uspekhi Mat Nauk* (translation: *Russian Mathematical Surveys*) **30**, 1–75 (1975)) in the Soviet Union.

The other area is fluid mechanics, where the elliptic umbilic suggested the design of the 'sixroll mill' (Berry, M. V. & Mackley, M. R. 'The sixroll mill: unfolding an unstable persistently extensional flow'. *Phil. Trans. Roy. Soc. (London)* **287**, 1–16 (1977)), a device for studying the effects of dissolved long-chain molecules on the flow of Newtonian fluid. The mill produces a sequence of flows with fully describable instabilities, and addition of polymer is dramatically revealed by changes in the topology of the pattern of streamlines. This specialised application has now been generalised (Thorndike, A. S., Cooley, C. R. and Nye, J. F. 'The structure and evolution of vector fields and other flow fields' (submitted to *J. Phys. A*)) into a comprehensive theory of flow patterns, which has already given insight into the structure of the geostrophic wind and the move-



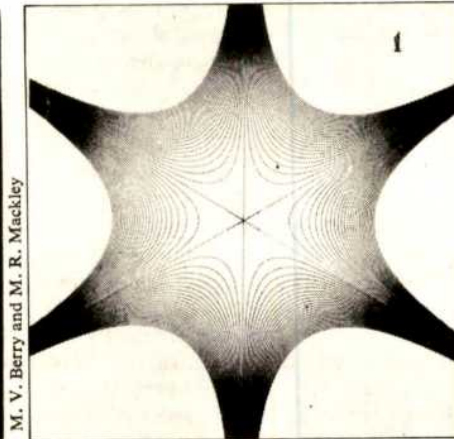
ment of ice in the Arctic ocean.

These are genuine applications of catastrophe theory; they have led to advances in our understanding of the physical systems concerned. It is important to distinguish them from illustrations of the theory, where the mathematics is employed correctly (that is to systems satisfying its axioms) but in more sophisticated derivations of results already known; elastic buckling and the mean field

theory of phase transitions fall into this category. The applications should also be distinguished from what I shall call invocations of the theory, where it is employed because of the suggestiveness of its images in the hope that its axioms might eventually be shown to apply; perhaps it is towards this area that Zahler's and Sussmann's criticisms are really directed.

MICHAEL BERRY

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M. V. Berry and M. R. Mackley

The six roll mill; experimental observations and computer simulations of the patterns illustrating elliptic umbilic critical point.

SIR,—While I do not wish to perpetuate unwarranted enthusiasm for the ability of catastrophe theory to transform the natural and social sciences, I believe that Zahler and Sussmann (27 October, page 759) have overstepped the bounds of decency in their vehement attack. Let me point out a few specific instances in which they seriously mislead the reader.

A fundamental error that they make is in the statement, "Catastrophe theorists agree that the term 'catastrophe' is reserved for certain kinds of singularity of smooth maps, seven of which have been described and classified elegantly by Thom". I can only conclude from this statement that Zahler and Sussmann have not read Thom's work. (There is no reference to Thom in the paper apart from his theorem). Thom describes the catastrophes which Zahler and Sussmann discuss as "elementary catastrophes" but repeatedly makes it clear that there are other kinds of catastrophe as well.

The authors' confusion on this point creates a straw man which they repeatedly flail in their article. For example, juxtapose the quote in the first paragraph of their paper with Thom's general definition of catastrophe and with their restricted one. The quote refers to a general approach to studying questions rather than the repeated use of a specific mathematical theorem, and makes much more sense in the context which was intended.

The section on 'Better alternatives' accuses catastrophe theory of ignoring the study of shock waves and bifurcation theory even though these are explicitly discussed in Thom's book, *Structural Stability and Morphogenesis* (Addison-Wesley, 1972), in which he lays out his theory in detail for the first time. I might add that one of the most successful applications of catastrophe theory has been to the study of shocks in a single convex conservation law (Schaeffer, D. 'Regularity theorem for conservation laws' *Advances in Mathematics* II 368–386 (1973) and Golubitsky, M. & Schaeffer, D. 'Stability of shock waves for single conservation law', *Advances in Mathematics* 16, 65–71 (1975)). In their portrayal of the scope and content of catastrophe theory, Zahler and Sussmann are simply wrong.

Let me turn to a second point. In a section entitled 'Careless discussion of evidence', Zahler and Sussmann quote Zeeman's statement: "Recent experiments by J. Cooke and T. Elsdale appear to confirm some of my predictions" (my italics). They then refute this statement with a quotation from T. Elsdale *et al.*, "we do not yet conclude that the observations here presented have confirmed Cooke and Zeeman's model to the exclusion of others" (my italics). The paper of T. Elsdale *et al.*, does indeed confirm some of the predictions of the Zeeman-Cooke model. It does not confirm the

model, but then Zeeman did not assert that it did. Zahler and Sussmann never say that these two statements contradict one another, but they clearly imply that Zeeman has made unsupported claims in his statement. This is false, and they try to mislead us into believing it.

At another point in their discussion, Zahler and Sussmann misrepresent the work of Kozak and Benham on denaturation of proteins. They assert that an essential feature of the work of Kozak and Benham is the 'delay rule' which predicts that there will be hysteresis in the denaturation-renaturation phenomenon. Yet Kozak and Benham do not rely upon this 'delay rule', and indeed use the 'Maxwell convention' throughout the second part of their three part work ('Denaturation: an example of Catastrophe II. Two-state transitions', *J. Theoretical Biology* 63, 125–149 (1976)). Zahler and Sussmann also criticise this model for predicting that the temperature denaturation curves have vertical slopes while the enthalpy change limits their steepness. Their criticism ignores the statistical discussion of Kozak and Benham which addresses the fact.

The thrust of this last criticism is also misplaced. A similar criticism could be made to the study of shocks in the solution of hyperbolic conservation laws, a phenomenon which is better understood mathematically. Real gases have viscosity, and viscosity prevents a truly discontinuous change in the velocity of the gas. Nonetheless, abrupt changes do occur. They can be modelled well by hyperbolic conservation laws which do allow discontinuous solutions. The addition of a term representing viscosity to the equations smooths out this discontinuity, and the behaviour of solutions has been studied as this viscosity term tends to zero. The conservation laws work well in giving approximations to real fluid flow. It is even the case, as we noted above, that catastrophe theory has described the shocks for the simplest (but only the simplest) conservation laws. The confusion of "the intuitive notion of 'jump' as a rapid change with the precise mathematical notion of a jump discontinuity" is not inherent to catastrophe theory, but is a common and useful approximation in many mathematical models.

I prefer to make a few remarks on the paper as a whole rather than continue to belabour specific failings. As a 'review article', Zahler and Sussmann review a single paper—their own. Their review is a summary and not a review. There are others who are more optimistic about the potential applications (and the past successes) of catastrophe theory than Zahler and



Sussmann are, but they have been ignored. Zahler and Sussmann have discovered that "catastrophe theory is a blind alley" because they have blinded themselves to anything hopeful that they might see. Surely a review of the "accomplishments of applied catastrophe theory" should not be so narrow. In particular, it should at least mention the work of J. M. T. Thompson on elastic stability (*Nature* **254**, 392-395 (1975)).

Zahler and Sussmann are also snide. They denigrate catastrophe theory for the "large number of (mostly unrefereed) publications praising each other extravagantly". This is an attempt at character assassination due to the fact that many papers are published in conference proceedings. I

think the remark is out of place—indeed I think the whole paper is out of place. It is deceptive, and it is not what it purports to be. There is much to be criticised in work which has been done using mathematical models based upon catastrophe theory, but the criticism of Zahler and Sussmann is merely mean spirited.

JOHN GUCKENHEIMER

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SIR,—My paper, to which Zahler and Sussmann refer, was presented as a discussion paper at a meeting of the New York Academy of Sciences. It was directed to a general audience at a time when catastrophe theory was not widely known. Since I could not presume a detailed knowledge of the theory or of biology, I intended to give

simplified explanations of both so that I might suggest possible uses of the theory in biology.

May I also suggest that one illustration of a catastrophic jump might be the apparent discontinuity in Sussmann's opinion of Zeeman's paper (in *Towards a Theoretical Biology* **4** (1972)). In 1975 Sussmann (*Synthese* **31**, 229 (1975)) writes "in most applications what is used is not catastrophe theory as a set of results, but catastrophe theory as a conceptual framework (for an example, see Zeeman's beautiful paper in *Towards a Theoretical Biology* on heartbeat and nerve impulse)". Now, writing with Zahler he expresses a very different opinion.

A. E. R. WOODCOCK

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## Censuring repressive regimes

SIR,—Every four years the International Union Against Cancer organises a huge international cancer congress, typically attended by perhaps 6,000 scientists. The next one is scheduled for 5-12 October 1978 in Buenos Aires, Argentina. However, in *Science* (21 October) and *Nature* (3 November, page 8) groups of scientists, including two recent Nobel prize-winners for medicine, have called on other scientists to boycott this conference because, it is claimed, "scientists, physicians, professors, journalists, intellectuals, and other (Argentinian) citizens have been arrested, imprisoned without benefit of *habeas corpus*, often tortured and sometimes executed without trial". These allegations are supported by editorials in *Nature* (**263**, 452; 1976 and **266**, 395; 1977).

We were telephoned by the press for comment on these letters, but could not comment because, like many other full time scientists, we were necessarily ignorant of conditions in most foreign countries. We therefore made contact with Amnesty International (AI), in the hope of securing some reasonably unbiased data. The 'Report of the AI Mission to Argentina' (1977) and the detailed letter which the Director of AI sent us appear to be objective and impartial, and the conditions they describe are really appalling.

Briefly, there was a military coup in March 1976. During the next six months about 2,000 Argentinians were killed by the government or (almost equivalently, it seems) disappeared. Killings and disappearances of non-violent Argentinians (a priest, for example, who said a requiem mass for a parishioner who had been killed by government agents) continue at a rate

of hundreds a month, and frequently bodies are discovered, some of people recently arrested, many with evidence of severe torture. Recent Argentinian laws explicitly forbid newspapers to report, comment on or make reference to abductions or the discovery of bodies, and also forbid propagation through any medium whatever of news or views with the purpose of lessening the prestige of the armed forces!

In the apparently impartial opinion of the Director of AI (personal communication, 25 October), "the state of affairs in Argentina is now one of the most serious in the world: Argentina has probably, since March 1976, the largest number of prisoners of conscience, disappearances and political killings in the whole of Latin America". (In most cases, according to AI, those affected were not terrorists.) However, even if, among many bad countries, Argentina is worst in this respect, it does not follow that a boycott is wise, for it is difficult to estimate the true probabilities of all its possible effects (especially if one is distracted by suppositions about the motives of the scientists who propose it or of those who oppose it).

It must be recognised by the proponents of any boycott that it is very unlikely to have much impact on the repression of non-violent citizens, while the opponents of a boycott must equally concede that there does exist a small probability that it would materially accelerate humanitarian progress. Balancing these considerations are the medical advantages of holding a cancer congress, but here a rather curious analogy exists; one single congress is very unlikely to have much impact on human cancer, but there does exist a small probability that it will materially accelerate medical progress. Moreover, because the Argentinian junta are apparently (*Financial Times*, 14 September) having difficulty,

because of their human rights record, in securing foreign investment and arms, it is uncertain which probability is smaller in this particular balance.

One must also ask, however, if scientific meetings are boycotted, where it will all end. In a related academic field, for example, the International Epidemiological Association will meet in Iran in 1979 and, overall, a large number of international scientific meetings take place in countries with governments which kill, or torture, or imprison non-violent citizens. If these several meetings were all avoided, so many different governments would thus be censured that none would thereby be singled out for special attention, and the net effect might merely be to forfeit the rather useful apolitical image which science has earned, while achieving little or no humanitarian progress. It might be practicable just to boycott those governments which persecute many scientists, but this seems rather artificial, and might merely aggravate anti-science sentiments. Moreover, the scientists who live in some of the countries concerned might much rather have a week or two of contact with the international community than suffer continued intellectual isolation. Perhaps, anomalously, boycotts do more harm than good in states with closed frontiers (unless the state would also restrict free attendance), but perhaps they are occasionally useful in states with open frontiers.

Advice and comments from other scientists, especially those who, unlike ourselves have actually worked under repressive regimes, would be timely, both about the general advisability or inadvisability of such boycotts (ignoring everybody's motives, please), and also about the specific 12th Cancer Congress in Argentina next year.

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# news and views

## Object Kowal, the most distant asteroid

from David W. Hughes

ASTEROIDS often get into the news. Only 2 years ago asteroid 1976 AA was found to be the first with a mean distance from the Sun less than that of the Earth. On 1 November this year slow-moving object Kowal was discovered and has subsequently been found to have the largest known orbit of any of the asteroids. It is orbiting the Sun between Saturn and Uranus.

Charles T. Kowal of the Hale Observatories, California Institute of Technology is carrying out a systematic survey of the outer solar system by photographing regions of the ecliptic using the 122-cm Schmidt telescope on Mount Palomar. He first spotted this strange asteroid when examining photographic plates that he had taken 2 weeks previously on 18 and 19 October. It was also discovered on the plate that had been taken by Tom Gehrels (University of Arizona) on 11 October.

Bodies in the Solar System appear to move across the sky against the backdrop of the vastly more distant, 'fixed' stars. A long-time exposure will thus produce a trail-of-light image for the Solar System objects as opposed to a point image for the stars. Also the positions of the objects change from one plate to the next. These trails can be detected by the use of microscopes.

Slow-moving object Kowal has an angular motion of only 0.0149 degrees per day, a speed scarcely greater than that of Uranus. As objects in the Solar System with near circular orbits have a mean daily angular motion proportional to  $a^{-3/2}$  where  $a$  is the semi-major axis of their orbit, this automatically means that object Kowal is in the outer reaches of the Solar System, near the orbit of Uranus. On 18 October it had a right ascension of  $2^h 05^m$  and a declination of  $+12^\circ 09'$ , placing it about half-way between Alpha Aries and Alpha Pisces. If the positions of the object on a set of dates are known,

its orbit can be calculated. Object Kowal has, however, moved only about three-quarters of a degree around its orbit since its discovery, thus making the orbital determination very inaccurate. Brian G. Marsden (Smithsonian Astrophysical Observatory, Cambridge) has published a preliminary calculation in Circular 3129 of the International Astronomical Union's Central Bureau for Astronomical Telegrams. This orbit is shown in Fig. 1. It has a low eccentricity, 0.031, a low inclination to the ecliptic,  $5.2^\circ$ , a peri-

helion distance of 15.836 AU, a semi-major axis of 16.340 AU, and a period of 66.1 years.

The object is extremely faint, having a visual magnitude between 18 and 19, this faintness being one of the reasons it has previously gone undiscovered. If it has the orbit shown in Fig. 1 its magnitude will change by only 0.5 over one period. There are thus very few telescopes capable of searching for such objects, those that can find them having to have an objective lens more than 100 cm in diameter.

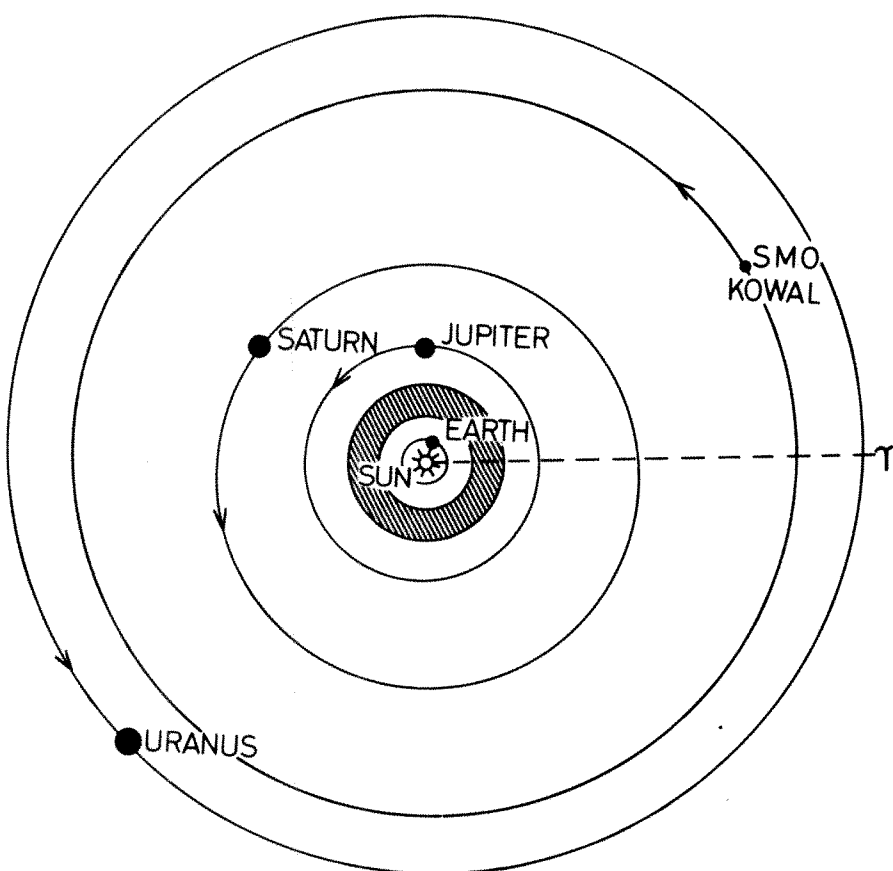


Fig. 1 The orbit of slow-moving object Kowal and the position of this new asteroid with respect to Earth, Jupiter, Saturn and Uranus. The hatched area is the main asteroid belt.  $\gamma$  is the first point of Aries.

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Even though object Kowal is faint, it is much brighter than any known comet would be at that distance. It has thus been concluded that it is an asteroid. It might even turn out to be the first of a new belt of asteroids orbiting between Saturn and Uranus. Most asteroids orbit the Sun in a broad belt between Mars and Jupiter (shown as a hatched area in Fig. 1). Some have larger orbits, Hidalgo for example has a highly eccentric orbit which approaches the orbit of Saturn. It must be remembered of course that the further away from the Sun and Earth an asteroid is, the more difficult it is to detect. The region between Saturn and Uranus could easily contain an Earth's mass worth of asteroids which have remained undetected until now.

The brightness of the asteroid can be

used to estimate the product of its albedo and its reflecting surface area. Asteroids seem to have albedoes ranging from about 0.035 to 0.27. As the albedo of object Kowal is unknown the size cannot be found accurately. It has been estimated to have a radius in the region 80–320 km. The largest asteroid, Ceres, has a radius of 380 km, and so object Kowal is definitely at the top end of the asteroidal mass scale.

Where do we go from here? More observations of object Kowal, stretching forward in time, or even lying unknown on photographic plates, will enable a much more accurate orbit to be calculated. Also now astronomers know that at least one asteroid, albeit a large one, is orbiting between Saturn and Uranus it is most likely that more will be discovered in the near future. □

## Workshop on hydrophobic effects

from Felix Franks

A workshop on Hydrophobic Effects was held at the 5th International Conference on Chemical Thermodynamics, Ronneby, Sweden, 23–26 August, 1977.

THE involvement of so-called hydrophobic effects in the maintenance of biological structures has been recognised for many years; G. S. Hartley first used the term in his analysis of micellar aggregation. The detailed nature of the environment of alkyl chains in water, and the manner in which their interactions give rise to supermolecular structures and promote the unique folding of biopolymers, are not yet too clear. Nevertheless, over the past 10 years a considerable effort has been devoted to studies of dilute aqueous solutions of alkyl derivatives. The workshop was arranged in an attempt to reach some agreement on terminology and symptoms of hydrophobic effects and to analyse those features which play a part in biochemical processes.

The question of suitable and correct standard states was discussed at length; for means of comparison, the transfer of an alkyl group from the ideal gas state to an infinitely dilute aqueous solution is the most useful process to consider, and the term 'hydrophobic solvation' was used to describe such a process. Although solution concen-

tration is commonly expressed in terms of mol fraction or molality, it was pointed out that the correct way of comparing solute environments in different solvents is through the molarity concentrations. This is also required for statistical thermodynamic treatments of solute-solute interactions, since the equations describing solution behaviour are referred to constant volume, rather than to constant pressure.

The standard thermodynamic functions of solvation which characterise the transfer of an apolar solute from the ideal gas to an infinitely dilute aqueous solution include a positive free energy which arises from a large, negative entropy, a negative volume and a very large, positive heat capacity. In fact, the limiting partial molar heat capacity of an alkane in aqueous solution is three times as large as the heat capacity of the alkane in its pure liquid state. Taken together with recent nuclear magnetic relaxation and computer simulation data, the heat capacities are consistent with the picture of a rotation of water molecules away from the alkane, with possibly slight deformations in the tetrahedral hydrogen bonding geometry, to give rise to cavities which can accommodate the hydrophobic groups. The hydrogen bonding regime which normally exists in liquid water is but slightly modified; whatever modification does take place is responsible for the negative entropy and the positive heat capacity of hydrophobic solvation.

Interactions between solvated alkyl residues can be studied at different

levels, ranging from pairwise solute effects to the complete removal of water and its substitution by a hydrocarbon. For the latter process the standard free energy of transfer is about  $-3.45$  kJ per mole  $\text{CH}_2$  groups. Intermediate between these two environments are the micelle, the phospholipid bilayer membrane and the interior of a globular protein. None of these three biologically important structures can be equated to the bulk hydrocarbon, and therefore free energy data relating to the transfer of alkyl groups from water to hydrocarbon should not be used for thermodynamic calculations of micellisation or protein unfolding. The hydrophobic pair interaction is considered to be an adequate model for enzyme-substrate interactions and for the contributions of exposed apolar amino acid residues to protein stability (or instability).

The thermodynamic functions describing such pairwise interactions are obtained as second virial coefficients from the limiting slope of the concentration dependence of the various excess functions. Recent excess volume ( $\Delta V^E$ ), heat capacity ( $\Delta C_p^E$ ) and compressibility ( $\Delta \kappa^E$ ) measurements on very dilute solutions indicate that these second virial coefficients have the opposite signs to those expected from the classical treatment of the hydrophobic interactions. Indeed,  $\Delta C_p^E$  is a very complex function of concentration and temperature. It seems that the hydrophobic pair interaction is qualitatively different from the interaction involving large numbers of alkyl chains, for example, micelle formation.

A comparison of gas and solution second virial coefficients of  $\Delta G^E$  shows that the interaction between pairs of alkyl groups in dilute aqueous solution is weaker than the corresponding interaction in the gas phase. Also, recent experiments with non-aqueous solvents (for example, dilute solutions of octane in N-methylacetamide or hydrazine) indicate the existence of a general solvophobic effect which differs from the hydrophobic effect in two aspects: it does not originate from a negative  $\Delta S^E$ , and it is not associated with a large positive  $\Delta C_p^E$ , both of which effects characterise the hydrophobic interaction. With rise in temperature water becomes more hydrophobic, and near  $150^\circ\text{C}$  its behaviour approaches that of 'normal' polar solvents.

So far only limited progress has been made in the molecular description of hydrophobic effects by means of suitable potential and radial distribution functions. Any useful interpretation of terms such as 'water structure making', commonly found in the literature, must await the development of credible radial distribution functions. In the meantime it is being recognised that

the 'interaction' between alkyl groups in water results primarily from repulsions between water and solute, and not from van der Waals attraction between solute molecules.

Most molecules for which hydrophobic effects are commonly invoked also possess one or more functional groups which contribute to the observed interactions in solution. In all cases such contributions of  $-\text{CONH}$ ,  $\text{CHOH}$ , and so on to  $\Delta G^\text{E}$  are negative, but only alkyl groups exhibit negative contributions to  $\Delta S^\text{E}$ . Even more important, cross interactions between alkyl and polar groups are not equal to the mean of the interactions between like groups. This finding has a bearing on calculations of protein stability in terms of amino acid side chain contributions.

Any future progress in our understanding of hydrophobic effects is not likely to come from thermodynamics, although there is still much scope for high precision measurements of excess functions in the low concentration range. Techniques which are showing promise include electron spin exchange between hydrophobic spin probes and the enhancement of nuclear magnetic relaxation by such paramagnetic spin probes. Monte Carlo and molecular dynamics simulations have also shown some promise, but in the absence of reliable potential functions it is questionable how much confidence can be attached to the results of such calculations. The same question mark hangs over recent computer simulations of protein dynamics, in which solvation and hydrophobic contributions to the stability of the folded protein were not, and indeed, could not be included. However, in view of the progress that has been achieved over the past 10 years in our general understanding of molecular interactions in aqueous solutions, the prospects for such ambitious ventures look quite encouraging.  $\square$



### A hundred years ago

THE chemists of Berlin have been occupied lately in analysing the wares of the wine merchants, and no little excitement has been caused by the discovery that the entire stock of one of the largest houses dealing in wines for medicinal purposes, consisted entirely of artificially prepared mixtures of spirit and sugar solutions, flavoured with various herbs.

From *Nature* 17, 29 November, 1877.

## Mechanisms of parasite immunity

from F. E. G. Cox

A meeting on Immunity in Parasitic Diseases: Antigens and Mechanisms of Immune Response was held under the auspices of the Institut National de la Santé et de la Recherche Médicale and the Institut National de la Recherche Agronomique with Professor André Capron as Chairman, at Grignon, France on 5-9 September, 1977.

PARASITOLOGISTS and immunologists need to meet from time to time to share their rapidly accumulating knowledge and there can be few better places to do so than a Louis XIII château. Twenty review papers and four round tables, each with a dozen or more contributors, demonstrated the healthy state that the science of parasite immunology has reached and for the first time various pieces of information painstakingly obtained in different laboratories began to fall into place. The two themes that came over time and time again were first that in each infection there are probably several effector mechanisms involved in immunity and that under particular circumstances any or all of these might be involved and, second, that eosinophils, macrophages, antigen-antibody complexes and complement may be more important to parasitic infections than hitherto suspected. Against this background it was possible to look forward as well as backward.

The initiation of any immune response begins with the antigens of the parasite. Most parasite antigens studied so far have been relatively crude extracts and Ruth Arnon (Weizmann Institute of Science, Rehovot) discussed the possibilities of obtaining pure antigens which could be characterised and synthesised. For some microorganisms this possibility is a reality and for many parasites there are indications that the isolation and characterisation of functional antigens has progressed sufficiently far for the synthetic stage to be contemplated. The isolation of such antigens depends on the exploitation of their characteristics such as enzymatic activity and ability to bind to drugs and ligands and P. Pery (Grignon) showed that this was not difficult to achieve. In the case of *Schistosoma mansoni* over 60 antigens have been identified. D. Bout and his colleagues at Lille, using drugs as

ligands, have found that malate dehydrogenase is a particularly important antigen in human *S. mansoni* infections and J. P. Rotmans (University of Leiden) has shown that only one isoenzyme of malate dehydrogenase is antigenic in mice. This antigen can be purified and used in microgram quantities in immunodiagnosis of schistosomiasis. An egg antigen described by J. Hamburger (Jerusalem) is the antigen responsible for granulomas in mice but also has considerable potential in immunodiagnosis as has a circulating polysaccharide antigen isolated by Y. Carlier and his coworkers at Lille. Other purified antigens also described include one from *Nippostrongylus brasiliensis* (A. Petit *et al.*, Grignon) and one from *Trypanosoma cruzi* which, as it is shared by both the blood and culture forms of this parasite, may be very useful as a potential vaccine (L. Hudson and D. Snary, Wellcome Laboratories, Beckenham).

The effector mechanisms attracted considerable attention particularly in the case of schistosomiasis. A. Capron (Lille) drew attention to the two main ways in which schistosomes are attacked, IgG and eosinophils and IgE and macrophages. The IgG involved is IgG2a and the activity of the eosinophils is enhanced by the presence of mast cells (Monique Capron *et al.*, Lille). The importance of eosinophils in immunity to parasites was discussed in general terms by A. B. Kay (Edinburgh) and in much more detail as the cells that actually attach to schistosomes and penetrate the body wall by C. Mackenzie *et al.* (National Institute for Medical Research, London) who pointed out that these cells adhere to other helminths as well. B. M. Ogilvie (NIMR) also drew attention to the similarities in the immune responses to parasites as different as *Elmeria* and *Nippostrongylus* living in the gut.

Macrophages are obviously important in immunity to parasites and their role in the production of nonspecific mediators of immunity was discussed by A. C. Allison (Clinical Research Centre, Harrow). Nonspecific immunity to blood parasites following the administration of *Corynebacterium parvum* or BCG was also discussed by F. E. G. Cox (King's College, London), who postulated a common mechanism for homologous, heterologous and non-specific immunity to malaria in rodents, and by B. Leblanc and J. C. Salmon (Villejuif). Another nonspecific mechanism, the activation of complement through the alternative pathway, was discussed by G. Hultdt (Stockholm) in the context of *Entamoeba histolytica*.

Immunodiagnosis and vaccination are the two main objectives behind immunological studies and the prospects

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for vaccination were discussed by D. S. Rowe (World Health Organisation, Geneva) but it is too early yet to assess the potential of purified or crude antigens as vaccines against the more important parasitic diseases. The successful vaccination of cattle and sheep against *Schistosoma bovis* using irradiated whole larvae was described by M. G. Taylor (St Albans) but a more cautionary story was told by G. M. Urquhart (University of Glasgow) who discussed the success of the irradiated *Dictyocaulus viviparus* vaccine and the lack of success with a similar *Ancylostoma caninum* vaccine which was due not to any defect in the vaccine but to the fact that American veterinarians preferred drugs which are very effective against this worm. For most of the parasitic diseases of man effective drugs are not currently available and until they are immunological research must not be allowed to decline; those present at the Grignon meeting will ensure that this does not happen. □

## Fly's eye view of cosmic rays

from K. J. Orford

AN interesting and elegant new experimental technique, designed to take 'pictures' of the tracks in the atmosphere of very high energy cosmic rays, has borne its first fruit. The results of a pilot experiment (Bergeson *et al.* *Phys. Rev. Lett.* **39**, 847; 1977) have demonstrated that such tracks may be observed using the very weak atmospheric nitrogen fluorescence light. Its significance is that the effective size of detectors of the very rarest cosmic rays has been increased by this technique to the volume of the atmosphere which can be seen from a point on the ground.

The main aim is to measure the energy spectrum of cosmic rays up to energies difficult to attain with any other technique. A continuous spectrum of energies is found to come from all parts of the sky up to an energy of about  $10^{20}$  eV (about 16 J) per particle. The cosmological problems centre on the sources of these particles, whether they are local or universal, and on how much such prodigious energies can be given to individual particles. If, for example, these cosmic rays are universal, then a limit is placed on the energy spectrum. At an energy near  $6 \cdot 10^{19}$  eV a proton will interact strongly

with the 2.7 K universal microwave background, and will lose a large amount of energy. The spectrum will then rapidly fall in the region of  $10^{20}$  eV. There are indications from current experiments that it does not (Cunningham *et al.*, *15th Int. Conf. on Cosmic Rays*, Plovdiv 1977), at least up to nearly  $10^{20}$  eV.

Just to detect the arrival of these cosmic rays is very difficult, mainly as a result of the small flux. A cosmic ray of energy greater than  $10^{20}$  eV falls on a square kilometre somewhere between once per year and once per century, such is the present uncertainty. Such a low rate is detectable only because the cosmic ray particle causes a shower of up to  $10^{11}$  electrons and other particles to build up along its track as it passes through the air. This shower has a lateral extent of a square kilometre or more, enabling particle detectors to be widely spaced on the ground to provide samples of the shower particles. For about 20 years, ever larger arrays of such detectors have been deployed around the world to sample large showers.

Some of the best information to date has come from the UK array at Haverah Park, near Harrogate. This has a sampling area of 12 square kilometres and has recorded showers of energy up to  $10^{20}$  eV. However, there is a limit to the size which such arrays can be made, the cost increasing with the area covered.

The new technique uses the whole atmosphere visible from any point as a detector. It exploits the fact that most of the energy of the primary cosmic ray does not reach the ground, but is dissipated as ionisation in the air. About 0.05% of this energy is released as nitrogen fluorescence light which produced a very short ( $\mu$ s) flash of light along the cosmic ray track. A detector designed to measure this flash was first proposed in 1965 (Greisen *Int. Cosmic Ray Conf.*, London, 1965). However, the technical problems are severe. The light output is very small and difficult to detect against the background light of the night sky (each electron in the shower gives only four fluorescence photons per metre of track).

The pilot experiment of Bergeson *et al.* involved the use of concave mirrors of 1.5 m diameter, each focusing a small section of sky onto the faces of 12 photomultipliers. Each tube views an area of sky about  $6^\circ$  across. Showers detected by a conventional particle array at Volcano Ranch, New Mexico, were compared with the images seen by the mirror assemblies and found to be in agreement. Following this, a full-scale experiment is being constructed on a mountain top in Utah, consisting of 67 mirror units. Thus a whole sky

image is obtained with a resolution of better than 0.007 steradians. This many-faceted experiment, early dubbed the 'Fly's Eye', should detect the highest energy showers at a distance of 50 kilometres. The effective collecting area of about 8,000 square kilometres should allow an energy spectrum to be measured with high statistical precision.

This would be enough by itself to justify the experiment, but other results of no less significance may be forthcoming. Because the track of ionisation is seen for most of its length, the point at which the primary cosmic ray particle first interacted with a nucleus in the air may be found. The observation of a large number of such points should reveal the mean free path of the primaries for interaction in air, and hence their atomic weight. The composition of high energy cosmic rays is at present an open question, and compositions other than a simple 100% proton flux bring their own problems concerned with production, acceleration and propagation from the sources to the Earth.

Finally, the mean free path of the primaries could enable the total cross section for nucleus-nucleus, and from that possibly proton-proton, interactions to be found at energies more than  $10^7$  times higher than those at present available. □

## Negative strand viruses

from T. Barrett

A meeting on Negative Strand Viruses and the Host Cell was held at King's College, Cambridge, from 1-5 August, 1977. The proceedings will be published by Academic Press early in 1978.

NEGATIVE strand viruses are responsible for many important human and animal diseases, including influenza, Lassa fever, measles, mumps, distemper, fowl pest and rabies. This group of viruses has in common the possession of a single-stranded RNA genome which is of opposite polarity to the messenger RNA (mRNA). One of the most striking points brought out at the meeting was the great advance that has been made in the understanding of the nature of the genome of these viruses, in particular the genome of the influenza A viruses which consists of

eight separate genes. Many speakers reported methods developed to assign functions to the individual genes. P. Palese (Mount Sinai School of Medicine, New York) is mapping gene functions of influenza B strains. Unlike the A strains, the B strains do not undergo dramatic antigenic shifts and do not cause pandemics. However, like the A strains they contain eight gene segments and eight virus-specific proteins can be detected in virus-infected cells.

The genome of the bunyaviridae was discussed by D. H. L. Bishop (University of Alabama). These are arthropod-borne viruses, some of which, for example California encephalitis virus, cause disease in humans. The genome consists of three segments; two of the segments code for two virus polypeptides while the third codes for two virus glycoproteins, probably through a polycistronic message.

The segmented nature of the influenza virus genome makes it an ideal candidate for sequencing studies because individual genes can readily be isolated. P. Fellner's group (Searle, High Wycombe) in collaboration with groups in Cambridge and Mill Hill, is sequencing the two smallest genes, 7 and 8. Both have a characteristic U<sub>6</sub>A sequence near the 5' end of the viral RNA (vRNA). In prokaryotes a similar sequence is associated with transcription termination (Rosenberg *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 717; 1976) but in this case at the 3' end of the transcribed RNA rather than at the 5' end of the template. It seems that the polyadenylated messenger complementary RNA (cRNA) of influenza virus is not a full length transcript of the vRNA and the extra sequences at the 5' end may be important as recognition sites for the viral replicase. In contrast, unpolyadenylated cRNA is a full length copy of the vRNA and is presumably the template for new vRNA synthesis (A. Hay, National Institute of Medical Research, London, and R. M. Krug, Memorial Sloan-Kettering Cancer Center, New York).

Negative strand viruses with unsegmented genomes seem to have only one promoter site, and the positions of the genes in relation to the promoter site have been determined in some cases using the ultraviolet transcriptional mapping technique. The sequences of the ribosome-binding sites of all the mRNAs of vesicular stomatitis virus (VSV) have been determined by J. K. Rose (Massachusetts Institute of Technology). All contain a single AUG initiation codon. The cap structure for the N, NS and L protein mRNAs was found by ribosomes in the initiation complex and showed a 9-nucleotide homology in the first 11 nucleotides extending from the 5' end. In contrast, the binding sites of the M

and G protein mRNAs differed both from each other and from those of the other mRNAs and did not include the cap structure. This observation has an interesting physiological aspect because translation of the M and G proteins is more sensitive to hypertonic shock (Nuss & Koch *J. Virol.* **17**, 283; 1976). A possible mechanism for this suggested by Rose is that, in the case of these two mRNAs, the ribosome needs to move to the binding site and this step may be sensitive to salt.

#### Transcription and translation

Genome transcription and translation was a major topic of the meeting. A. K. Banerjee (Roche Institute, Nutley, New Jersey) proposed that synthesis of a leader RNA sequence before any mRNA synthesis, as described for VSV and Newcastle disease virus (NDV), may be a general strategy for the initiation of RNA synthesis by negative strand viruses with unsegmented genomes. All leader RNAs studied are approximately the same size and their compositions are similar. However, hybridisation studies show that there is virtually no sequence homology. The 3' end of the VSV genome has a high U content and more than half the leader sequence is composed of A residues. This probably functions as a recognition site for the virion-associated RNA polymerase. These virus genomes can be transcribed efficiently *in vitro* using a virus-associated polymerase.

A. J. Hay, T. Barrett (University of Cambridge) and J. M. Taylor (Institute for Cancer Research, Philadelphia) all found evidence for transcriptional control of individual cRNA species during influenza virus replication. Hay found that the amount of unpolyadenylated cRNAs, presumably the templates for vRNA synthesis, were produced in roughly equal amounts for each segment while the polyadenylated cRNAs, presumably the mRNAs, were produced in different amounts for each segment. Barrett and Taylor both described the preparation of cDNA copies of influenza virus RNA made using reverse transcriptase, which are being used to study the time course of vRNA synthesis.

J. Content (Institut Pasteur, Brussels) described a coupled cell-free transcription/translation system for influenza virus capable of producing authentic virus proteins *in vitro*. The proportion of viral polypeptides obtained *in vitro* was consistent with translation of primary RNA transcripts.

L. A. Ball (University of Connecticut) reported that six virus-specific gene products of NDV were faithfully expressed when both transcription and translation were carried out *in vitro*.

Ball also used the ultraviolet transcription mapping technique to determine the number of promoter sites and the gene order of NDV. The results were consistent with a single promoter site and a gene order of 3'-NP-F<sub>0</sub>-M-HN-L.

The assignment of virus cores to the conflicting demands of virus assembly and RNA synthesis was the subject of a stimulating talk by D. W. Kingsbury (St Jude Children's Hospital, Memphis). He has isolated two metabolically distinct virus core populations, free and bound, in Sendai virus. The bound fraction, inactive in RNA synthesis, contains the newly assembled cores and is likely to be the source of both the cores that enter the virion and those that become active in RNA synthesis. The free cores, active in RNA synthesis are an older population. Kingsbury suggested that a maturation process whereby more P and L protein is added to the core determines its assignment to the free RNA synthesising fraction and that once a core engages in RNA synthesis it is committed to remain within the cell and cannot be encapsidated.

H. Lodish (Massachusetts Institute of Technology) reported on the glycosylation of the virus glycoproteins at different stages in their journey from the rough endoplasmic reticulum to the cell membrane, from which they are finally incorporated into the virion. The unglycosylated form (G<sub>0</sub>) could only be detected by translation *in vitro*. A partially glycosylated form (G<sub>1</sub>), lacking the terminal sialic acid, could be detected on the rough endoplasmic reticulum, while the fully glycosylated form (G<sub>2</sub>) was detected on the smooth endoplasmic reticulum, the plasma membrane and in the mature virion.

#### Virulence

One of the most interesting sessions of the meeting was concerned with aspects of virulence. Although this is a very active area of research, no clear picture of the molecular mechanism underlying virulence has emerged. H. D. Klenk (Institut für Virologie, Gießen) reported that NDV strains differ with respect to cleavage of their glycoproteins and these differences are an important factor underlying variations in NDV pathogenicity. With virulent strains of NDV, cleavage and production of infectious virus occurs in all host cells analysed, while with avirulent strains this is the case only in a few host systems. However, with influenza virus, although cleavage of the glycoproteins is indispensable for the formation of infectious virus particles, other factors also influence virus virulence. R. Rott (Giessen) suggested that a gene constellation determines virulence in the influenza virus because, as a rule,



infection with recombinants with multiple gene exchanges produces less severe clinical illness. A multigenic determination of virulence was also favoured by J. L. Schulman (Mount Sinai School of Medicine, New York) who found that in random recombinants of a low yielding and a high yielding strain of influenza virus the genes for proteins NP, M and NS were selected in recombinants which replicated in high titres while no such selection for P genes was observed. W. J. Bean (St Jude Children's Hospital, Memphis) found that virulent recombinants always contained the NP gene from the virulent strain and, in contrast to Schulman's findings, gene 3 which codes for one of the P proteins. J. Oxford (National Institute for Biological Standards, London) isolated a clone derived from recombinants of virulent and avirulent human influenza strains which contained all the genes, except the two coding for the surface glycoproteins, from the avirulent parent but which was virulent for man. It seems that some fine coordination exists between the genes for the internal proteins of influenza virus whose balance can be upset either by mutation or recombination. This set of genes is in turn dependent on the two surface antigens for its expression.

#### Defective particles

Defective interfering virus particles are aberrant virus particles which lack parts of the virus genome and are produced when virus is passaged at high multiplicities. These DI particles interfere with the replication of normal virus particles and could be a host defence mechanism. That a host function is required for DI particle formation was shown by C. Y. Kang (University of Texas, Dallas) who found that VSV DI particles could not be produced in cells pretreated with actinomycin D, while if present in the inoculum DI particles can be replicated in the presence of the drug. DI particles can be derived from either the 5' or 3' ends of the non-defective genome. Those derived from the 5' end are capable of only a very limited RNA synthesis *in vitro* and are thought to inhibit the non-defective virus at the replicase level. J. Perrault (University of California, San Diego) and D. Kolakovsky (University of Utah, Salt Lake City) described DI particles derived from the 5' ends of VSV and Sendai virus, respectively, with inverted complementary terminal sequences. These inverted complementary sequences at the 5' end presumably confer a replicative advantage on the DI particles, since synthesis of both plus and minus strands could be achieved by the same replicase which normally recognises the

sequences at the 3' ends of the minus and plus strands.

R. A. Lazzarini (National Institutes of Health, Bethesda) described another type of DI particle of VSV derived from the 3' end of the genome. This DI particle has a leader RNA sequence and the genome is transcribed into 12–17S mRNA *in vitro* and *in vivo* without a helper virus. It lacks the greater part of the L-protein gene which is nearest the 5' end of the non-defective genome. In mixed infections, in addition to interfering with the replication of the infectious virus, it can augment the production of virus proteins, a phenomenon which may in some way trigger an enhanced immune response by the host. Clearly DI particle formation and its mode of interference is a complex process and one which will become increasingly important from the point of view of their possible role in the treatment of virus diseases. □

## Nuclear radii and magnetic moments by laser spectroscopy

from P. E. Hodgson

THE charge and matter distributions of nuclei, and their magnetic moments, are important features of nuclei, and many efforts are being made to determine them with increasingly high precision. It is often instructive to see how they change through a series of isotopes of the same element, but such studies are limited by the availability of the separated isotopes in sufficient quantities for accurate measurement. This makes it desirable to develop methods that are applicable to very small quantities of the isotope, so that measurements can be carried out on rare and on radioactive isotopes.

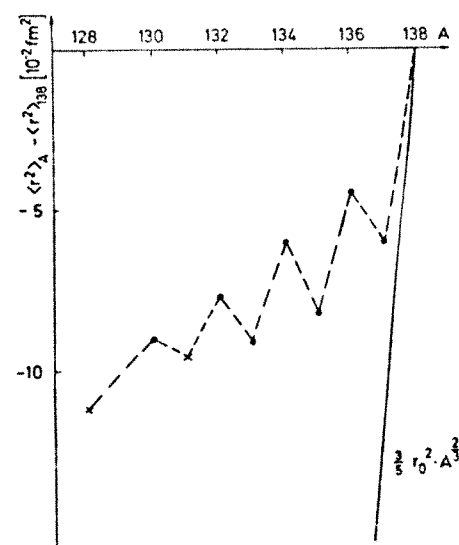
One of these methods uses high resolution laser spectroscopy, and the results of a determination of the nuclear radii and magnetic moments of  $^{129}\text{Ba}$  and  $^{131}\text{Ba}$  in this way has recently been reported by Nowicki and colleagues from the Nuclear Research Centre at Karlsruhe (*Phys. Rev. Lett.* **39**, 332; 1977).

The isotopes of barium were produced by (d,xn) reactions and subsequent  $\beta$ -decay from enriched  $^{130}\text{Ba}$  and  $^{134}\text{Ba}$ . The isotope  $^{131}\text{Ba}$  was also produced by neutron irradiation of enriched  $^{130}\text{Ba}$  in a nuclear reactor. The isotopes were enriched by an electromagnetic mass separator. This produced between 1 and 10 ng of the

isotope, sufficient for the laser method. The sample was in each case placed in an atomic-beam oven to produce a collimated beam of atoms. There is a second reference beam using the natural isotopic mixture. Light from dye lasers is passed through the atomic beams and this enables the hyperfine structure of a selected transition to be determined.

In this work the hyperfine structure of the 553.6 nm resonance transition  $6s^2\ ^1S_0-6s6p\ ^1P_1$  was measured by resonance fluorescence. The frequency of this transition, and indeed of all transitions, is slightly affected by the spatial extent of the nuclear charge distribution, and a quantum mechanical analysis shows that the shift in the frequency is proportional to the mean square radius of the charge distribution. The change in frequency from one isotope to another is thus proportional to the difference between their mean square radii.

This experiment gave for the mean square radius difference between barium 131 and 130 a value of  $-0.006\text{ fm}^2$  and between barium 130 and 128 a value of  $0.022\text{ fm}^2$ . These results are shown in the figure, together with corresponding values for other isotopes. This shows that the general trend of the charge radii continues smoothly for the even isotopes and that the large odd-even staggering is markedly reduced at neutron number 75. The magnetic moment can also be calculated from the hyperfine splitting, and a value of  $-0.714\ \mu_N$  is found for  $^{131}\text{Ba}$ . □



Differences of mean square radii of the barium isotopes as obtained from optical isotope shifts. The straight line shows the dependence to be expected from a standard homogeneous sphere.

# review article

## Study of membrane permeability changes by fluctuation analysis

Charles F. Stevens\*

*The molecular basis of the changes in membrane permeability that underlie the nerve impulse can be studied by statistical analysis of the random fluctuations in current through the nerve membrane. Such "noise analysis" has already begun to clarify the nature of the gating mechanisms that control the flow of ions across the membrane at the neuromuscular junction.*

TWENTY-FIVE years ago this year Hodgkin and Huxley published a series of papers that have served as the basis for virtually all succeeding studies on the mechanism of the nerve impulse<sup>1,2</sup>. The key idea in their analysis is that the permeability of the nerve membrane to certain specific ions, such as Na and K, is controlled by the voltage difference across the membrane. Cells expend metabolic energy to keep the concentrations of the various ions within the cell different from the concentrations in the outside bathing medium—for example, the intracellular concentration of Na is about an order of magnitude lower than it is in the extracellular fluid bathing the cell—and Hodgkin and Huxley showed that the electrical signs of the nerve impulse are accounted for by the permeability changes to these ions, and the resultant ionic rush toward equilibrium. To understand the molecular basis for nervous activity, then, we must penetrate the mechanism by which cell membranes change their permeability to ions.

Despite considerable progress in our understanding of neuronal mechanisms in the past 25 years, we still do not know precisely how, at the molecular level, membranes can alter their ionic permeabilities. Quite recently—during the past five years—several new approaches to this problem have been developed, and the expectation of their success has given rise to an optimism in the field that we may soon discover the molecular basis for the nerve impulse. My goal in this review is to describe one such approach, fluctuation analysis.

The idea behind fluctuation analysis is to exploit the inherently probabilistic nature of the processes that underlie the membrane permeability changes in order to gain information about the molecular mechanisms responsible. The functioning of excitable membranes depends on the opening and closing of channels. Because these channels behave probabilistically, the exact number of open channels fluctuates around the average value. For example, if a membrane contains 1,000 independent channels, each of which has a probability of being open of 0.5, the average number of open channels will be 500. A particular membrane might actually have 490 open channels at one time, 514 at a later time, and 502 at a still later time. These fluctuations in the number of open channels are governed by the same physical processes responsible for all channel behaviour, so the study of the statistical structure of the fluctuations can give information about the underlying biophysical mechanisms. It turns out, in fact, that fluctuations can sometimes contain information that is inaccessible by other practical techniques. I shall try here to explain, in largely non-mathematical terms, how fluctuation analysis has been used to increase our understanding of membrane per-

meability mechanisms, and shall indicate how the technique can contribute in future studies. Additional information on this subject can be found in a non-mathematical survey<sup>3</sup>, and in several more technical review articles that have appeared recently<sup>4-6</sup>.

My explanations about fluctuation analysis will be chiefly in the form of specific examples, first drawn from investigations of synaptic channels that are caused to open by the neurotransmitter acetylcholine, and second from the nerve membrane itself. Before giving these examples, however, I shall review briefly current views about the molecular basis for the nerve impulse, and survey some standard techniques for characterising fluctuations.

### Channels and gates

Changes in permeability to ions are responsible for the nerve impulse. How do these changes occur? The current view is that nerve cells have intrinsic membrane proteins that control the permeability to specific ions by providing channels through which they can pass. Each channel type is named for the ion that normally best passes through it, so one speaks of "sodium channels" and "potassium channels", even though potassium ions can move, for instance, through "sodium channels" to some extent.

Understanding how these membrane proteins regulate permeability to ions depends on the answers to two central questions. The first is: what are the molecular mechanisms responsible for the selective permeability of channels? For example, what is the physical basis for an ion-channel interaction that permits potassium ions (crystal radius = 1.33 Å) to pass through and nearly excludes sodium ions (crystal radius = 0.99 Å). The second central question relates to mechanisms by which membrane proteins alter ionic permeability. How, for example, is a membrane able to increase its permeability to sodium ions by many orders of magnitude in less than a millisecond? Questions of this second sort are said to deal with channel "gating", because increases in permeability occur as if gates obstructing the flow of ions through channels suddenly opened. This review is concerned primarily with gating rather than with selectivity.

Over the years since Hodgkin and Huxley first studied gating of sodium and potassium channels in the squid giant axon considerable information about this process has accumulated. There is now general agreement that gating occurs by conformational changes in a macromolecule. These conformational changes are driven principally by interactions of the gate with the membrane electric field (membrane field strengths change by about 100 kV cm<sup>-1</sup> during the nerve impulse) in electrically excitable membranes such as those of nerve cells, or by the binding of a ligand known as a neurotransmitter in chemically activated membranes such as those that occur at synapses. Ultimate

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understanding of gating would then involve knowing how electric fields or ligands can induce conformational transitions, knowing the number of possible conformational states the gate can assume and the rates of transition between these states, and finally relating this information to the structure of the gating macromolecule.

One of the main approaches to understanding the behaviour of gates is through the study of kinetics. This approach was instituted by Hodgkin and Huxley and involves measuring the rates at which channels go from the open to the closed state in various circumstances. For example, one might drive channels from the closed to the open state by causing a step-like change in the voltage across the nerve membrane. The time course of opening would be monitored by measuring the permeability of the membrane to, say, sodium ions as a function of time after the voltage step. One then tries to make inferences about the nature of the underlying conformational changes from the kinetics of channel opening revealed in such an experiment. Usually, experiments measure only the average number of channels open as a function of time in the sort of experiment just described, but more recently membrane biophysicists have been studying the fluctuations around the mean number of open channels. Such fluctuations occur because channel opening and closing is a probabilistic process. Although the investigation of these fluctuations is merely a variation on the more usual kinetic analysis, additional information can sometimes be gained from the fluctuations. My purpose here is to indicate why this is so.

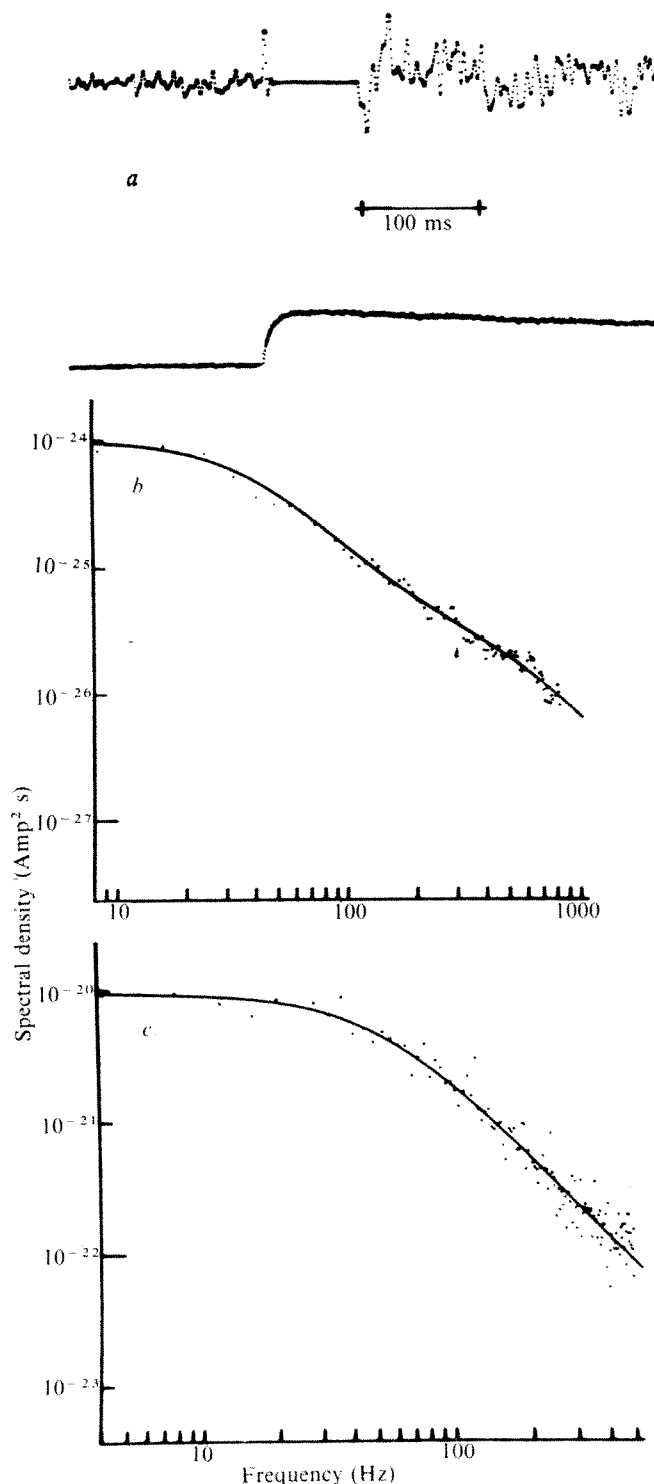
### The structure of noise

Records of a fluctuating quantity, such as the number of open channels, have a statistical structure, and information about a system can be inferred from the character of this structure. Before giving examples of uses of fluctuation analysis, then, I shall summarise briefly some standard characterisations of noise. The following discussion will usually assume that the systems under study are stationary. This means that the statistical properties of the fluctuations do not change with time over the course of a set of observations. For example, if the fluctuating quantity under study is the number of open channels, the exact number of channels open varies from instant to instant, but the average number open must stay fixed for the length of the sample; if the average number of channels open is  $1/2$  at the start of the observation period, an average of  $1/2$  must be open throughout the period.

The simplest characterisation of fluctuations is the variance, that is, the average of the squared deviations from the mean. The variance is a usual measure for the amplitude of fluctuations.

The variance indicates the overall magnitude of fluctuations, but gives no information about how rapidly they vary with time. Random signals can, however, differ dramatically in how fast the wiggles occur with, for example, one type of system producing a signal dominated by low frequency fluctuations and another by higher frequencies. Much of the information about a system that can be extracted from its fluctuations is contained in this frequency structure. A natural way to extend the simple characterisation by variance to give information about the rapidity of fluctuations is to find the contribution that each frequency component of a random signal makes to the overall variance. A record of fluctuations could be decomposed by Fourier analysis into its various frequency components (for example, 1 Hz, 2 Hz, 3 Hz, . . . . ., 100 Hz), and the variance per Hertz contributed by each component could be computed. If the random signal generated by the system were slowly varying, the variance contributed by the higher frequencies would be small, whereas a rapidly changing signal would have a much larger contribution from the high frequency components. The collection of numbers that specify the variance per Hertz for the different frequency components of a random signal is known as the spectrum associated with that signal. Figure 1 presents examples of two experimentally determined spectra displayed in the usual double logarithmic plots.

The individual record of fluctuations produced by a probabilistic system is almost never exactly the same twice, but the



**Fig. 1** Sample fluctuations, and spectra from nerve and synaptic membranes. *a*, Fluctuations in potassium current and mean potassium current (proportional to potassium conductance) from a voltage-clamped frog node of Ranvier (T. Begenisch and C.F.S., unpublished observations). Lower trace is mean current through potassium channels as a function of time, with the potassium conductance activated by a positive going voltage step. Upper trace is simultaneous recording at  $40\times$  greater gain with the mean current subtracted out. Fluctuations on the left of the upper trace represent equipment noise contributions. Fluctuations due to channel opening and closing are visible on right of trace after the period of flat base line which represents a blanking pulse to suppress filter transients. *b*, Spectrum of frog node of Ranvier potassium current fluctuations such as those in *a* (from T. Begenisch and C.F.S., unpublished observations). *c*, Spectrum of fluctuations in current through acetylcholine activated channels at the frog nerve-muscle junction (C. Lewis and C.F.S., unpublished observations). Acetylcholine was iontophoretically applied to a voltage clamped post-junctional membrane, and the resulting current fluctuations (proportional to conductance fluctuations) Fourier analysed to give the spectral densities as a function of frequency plotted above.

spectrum characterising the fluctuations is, except for sampling errors, always just the same. This spectrum reflects the characteristic times of the underlying molecular processes, and in fact the reason for determining a spectrum is that it gives a way of measuring these characteristic times. If underlying processes are fast, the spectrum will reveal large contributions from high frequencies, and if the system is sluggish, only low frequencies will be appreciable.

Probability theory provides standard recipes for calculating what spectrum any underlying probabilistic mechanism will produce<sup>7,8</sup>. If one can formulate a probabilistic theory for a process, that theory provides predictions not only about the system's average behaviour, but also about the spectrum associated with the fluctuations about this average. Comparing predicted spectra with observed ones thus provides a way of testing for the accuracy of some proposed mechanism. It is extremely important to emphasise that no automatic way exists for using a spectrum to gain information about a system. The study of fluctuations can, as will be explained later, give information that might otherwise be inaccessible for practical reasons, but all inferences depend entirely on some sort of theory about the source of the fluctuations.

### Synapse channels

Fluctuation analysis has been most extensively used to study the permeability changes produced by the application of the neurotransmitter acetylcholine to postsynaptic membranes, and this system will thus be used for the first examples. Although the examples presented here do not by any means include all of the applications of this technique, they do illustrate types of use. Application of fluctuation analysis to postsynaptic membranes has recently been reviewed<sup>6</sup>.

When a nerve impulse reaches a synaptic nerve terminal on a muscle it causes the release of acetylcholine from the nerve ending. The acetylcholine diffuses to a specialised postjunctional region of the muscle membrane where it causes a transient permeability increase to sodium and some other ions. The permeability to these ions increases very rapidly and then decreases again over a several millisecond period. These permeability changes are the first step in a sequence that leads to muscle contraction, and they can be measured by recording the currents that flow through the muscle membrane. These currents that reflect the permeability increase caused by acetylcholine are called endplate currents.

Until fairly recently, workers in the field had generally believed that the endplate current was a fairly faithful representation of the time course of acetylcholine concentration at the postjunctional membrane. Acetylcholine is removed from the vicinity of the postjunctional membrane by diffusion and enzyme action, and the assumption had been that it took several milliseconds for the acetylcholine to disappear by these processes and that endplate current continued to flow for as long as the acetylcholine was present. An analysis of the acetylcholine-produced permeability increase at the frog neuromuscular junction led Magleby and Stevens<sup>9</sup> to propose a theory that supposed the rate limiting step in channel closing is a conformational change in the gating molecule rather than a decline in acetylcholine concentration. According to this theory, one must assume that acetylcholine remains near the postjunctional membrane only for a very short time. During the brief action of acetylcholine, channels open and then close probabilistically over a several-millisecond period after the acetylcholine has been removed. This theory accounts quantitatively for many properties of the endplate current, but the assumption that the channel-closing conformational change is rate limiting could not be put to direct test with the techniques available. The difficulty was that the acetylcholine concentration at the postjunctional membrane could not be rapidly measured or controlled. This difficulty was circumvented by using fluctuation analysis.

Katz and Miledi had discovered that sizeable fluctuations in muscle membrane permeability occur when a constant concentration of acetylcholine is present at the neuromuscular

junction, and they concluded that these fluctuations represent the random opening and closing of channels<sup>10</sup>. The scheme Magleby and Stevens developed to describe how acetylcholine causes permeability increases, being inherently a probabilistic theory, predicted the spectrum of these fluctuations. Specifically, channels were assumed to have just two conductance states, open and closed, and a channel was viewed as fluctuating randomly between these two states when acetylcholine was bound to a specific receptor associated with the channel. This theory predicts<sup>9</sup> that the spectral density  $S(f)$  (contribution to the total variance) of the component of fluctuations with frequency  $f$  should be given by

$$S(f) = \frac{4\gamma g/\alpha}{1 + (f/2\pi\alpha)^2} \quad (1)$$

where  $g$  is the average conductance increase caused by acetylcholine,  $\alpha$  is the average rate at which open channels make the transition to the closed state, and  $\gamma$  is the conductance of a single open channel. The average conductance can be measured in the same experiment that produces records of fluctuations, and  $\alpha$  can be obtained through the Magleby-Stevens theory, independently of the fluctuation measurements, from endplate currents produced by nerve stimulation. The only parameter in this equation not found independently was the single channel conductance  $\gamma$ , and this was known to have an approximately constant value under a variety of experimental conditions that produced quite large changes in  $\alpha$  and  $g$ .

The strategy then is to observe the conductance (that is, effectively permeability) fluctuations and calculate their spectrum. If the theory is correct, equation (1) should describe the spectrum accurately with only one constant  $\gamma$  to be estimated from the fluctuations. As various conditions are changed that alter the spectra,  $\gamma$  should remain constant. If the channel closing step is not rate limiting, or if the theory is incorrect in a number of other ways, equation (1) should not accurately characterise the experimentally determined spectra under the various different experimental conditions.

Using this approach, Anderson and Stevens<sup>11</sup> found that equation (1) was indeed an accurate description of the spectra. This success of the theory gave strong support to the idea that channel gating properties, rather than loss of acetylcholine from the nerve-muscle junction region, are rate limiting during the normal endplate current. Furthermore, once the theoretical interpretation of the source for fluctuations could be accepted, these same experiments gave a measurement of  $\gamma$ , the single channel conductance.

Two properties of individual channels may thus be estimated from fluctuations in permeability through the use of equation (1), the single channel conductance  $\gamma$  and the average closing rate for open channels  $\alpha$ . The statistical theory that led to equation (1) provides some additional information about the channel behaviour: the average length of time  $\tau$  that a channel, once open, remains open is just  $1/\alpha$ . Fluctuation analysis therefore gives a way of measuring the conductance of a channel, and the mean length of time it stays open without ever actually making measurements on a single channel.

Generally various inevitable sources of instrumentation noise make it impossible to record directly, and in isolation, single channel currents. By carefully analysing the sources of noise in current measurements and cleverly designing apparatus that minimises these noise contributions, however, Neher and Sakmann<sup>12</sup> have recently been able to record the currents flowing through individual acetylcholine-activated channels in frog postjunctional membrane. Their results are in accord with the picture developed on the basis of fluctuation analysis: channels seem to have only two conductance states (open and closed), and the duration of the open state is exponentially distributed. The mean open time and  $\gamma$  have values in agreement with those determined by investigation of fluctuations arising from many channels independently opening and closing. Neher's and Sakmann's achievement is particularly important because cer-



tain assumptions made in the theory on which fluctuation analysis is based could not be independently checked. For example, all of the data obtained before their direct demonstration of simple open-closed behaviour were equally consistent with the idea that a channel opened instantaneously and then decreased its conductance exponentially with time.

Fluctuation analysis has been used to answer a number of questions about acetylcholine-activated channels<sup>6</sup>. In some instances, the quantities of interest were single channel conductance or mean open time and equation (1) had to be used to estimate these quantities because they could not be found by alternative means. In other instances certain experimental manipulations led to more complicated situations in which the spectra had a more complex form than equation (1) and an equation for the spectral density had to be derived from an analysis of mechanisms. I will give two specific examples that illustrate these approaches.

Colquhoun *et al.*<sup>13</sup> have recently used fluctuation analysis as an aid in studying acetylcholine metabolism at the rat nerve-muscle junction. Their approach depends upon the important discovery by Katz and Miledi<sup>14</sup> that various analogues of the natural neurotransmitter acetylcholine each cause the channel to remain open for a different mean time for a given set of conditions. For example, the mean open time is shorter for carbacol than for acetylcholine, and longer for suberyldicholine than for acetylcholine. Colquhoun *et al.* found conditions under which monoethylcholine would be taken up by the nerve terminal and synthesised into acetylmonoethylcholine. This "false" neurotransmitter was then released by the nerve on stimulation. The fact that acetylmonoethylcholine was being released could be detected because channels stayed open a shorter average time with this false transmitter than with the normal acetylcholine, and the false transmitter was less potent than the normal one. The characteristic channel behaviour elicited by the false transmitter was found by using fluctuation analysis to measure channel mean open time and single channel conductance for externally applied acetylmonoethylcholine. Being able to detect the quantity of false and natural transmitter released means one can study the details of vesicle filling with more precision than otherwise possible.

Ruff<sup>15</sup> has recently used fluctuation analysis to help unravel the action of local anaesthetics at the postjunctional membrane. Normally the endplate current decays according to a simple exponential time course (reflecting the conformational change in closing) but, after treatment with local anaesthetics, the decaying phase of the endplate current exhibits two main exponentials, one faster and one slower than normal. Of the various hypotheses that could account quantitatively for this behaviour, two specific ones were: (1) the local anaesthetic molecules alter the lipid microenvironment of receptors and cause islands of lipid and proteins with two different characteristics. Some islands are stiffer than normal, whereas others are more fluid. In the more fluid islands, the conformational change can occur more rapidly (this gives the rapid exponential decay), whereas the stiffer environment produces slower than normal conformational changes (this gives the slower exponential component in the decay of endplate current). (2) Local anaesthetic molecules could rush in and block open channels so that ion transport through the membrane could not proceed but nor could the conformational change occur that closes the gate. In this theory the rapid exponential in the decay would reflect the initial establishment of a steady state number of blocked channels, and the second exponential would arise from the slow unblocking and closing step.

Both of these theories, as well as others, could account quantitatively for the decay of endplate currents and no observation on mean endplate currents can distinguish between them because they give rise to the same descriptive equation. The theories do, however, make different predictions about the spectra. Both mechanisms would give a spectrum with two components that is more complicated than the type described by equation (1). The amplitudes of the components are different for the two theories, so that a measurement of the mean endplate current and the spectrum permits a differential test. Ruff has carried out this procedure for the two theories indicated above

and others, and has found that the blocking mechanism is in quantitative agreement with the experimental observations, but predictions of alternative theories are not. This example illustrates a fairly common occurrence: different molecular mechanisms make identical or practically indistinguishable predictions about average behaviour, but give rise to spectra with easily detectable differences.

## Nerve channels

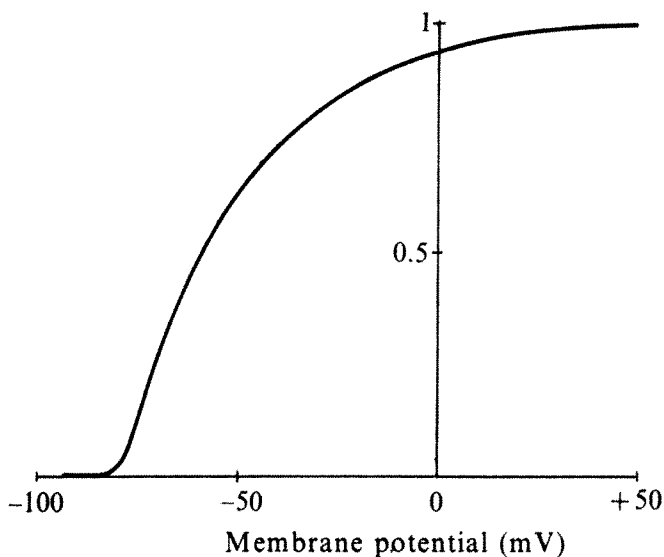
In 1966 Verveen and Derksen published a short account of their studies on fluctuations in nerve membrane permeability<sup>16</sup> and thus began the use of fluctuation analysis to investigate nerve membrane properties. Interestingly, the main motivation for the early studies from Verveen's laboratory related more to information processing properties of the neurones than to the molecular mechanisms of excitability. Verveen's thesis<sup>17</sup> had been concerned with random threshold fluctuations in nerve, and he and Derksen had set out to discover the membrane correlates of this phenomenon. Blair and Erlanger<sup>18</sup> had, in the course of their pioneering work on the nerve impulse, discovered that nerves stimulated near threshold produce nerve impulses probabilistically, and Pecher<sup>19</sup> had quite carefully investigated this phenomenon and found that the threshold variations were independent and normally distributed if stimuli were applied at an appropriate rate. Early interest thus centred on the probabilistic behaviour neurones exhibited in some circumstances, and was largely motivated by implications this behaviour has for nervous system function: how can the nervous system be reliable when its basic elements, the neurones, are "unreliable" in the sense that they cannot be counted on to give a nerve impulse whenever stimulated?

In recent years, however, the main motivation for the study of fluctuations in properties of the nerve membrane has been related not to information processing, but rather to the significance for the molecular basis of nerve excitability. Furthermore primary interest has shifted from studies of the so called "1/f noise" first described by Verveen and Derksen to the conductance fluctuations that arise from the probabilistic opening and closing of channels. In the remainder of this review I shall concentrate on "channel noise" rather than on the 1/f noise associated with ion motion through open channels; that is, my attention will be limited to fluctuations in conductance associated with the opening and closing of the channels responsible for nerve excitability.

A resting nerve cell normally has an interior that is about 60 mV more negative than the outside solution. If the voltage across the cell membrane is displaced from the normal resting value of -60 mV to more positive values, ion selective channels (Na, K, Ca) are caused to open so that the membrane conductance increases. Hodgkin and Huxley discovered that the increase in conductance as a function of voltage is a sigmoid function of voltage for each channel type (Fig. 2).

Two limiting mechanisms can be envisaged for this type of behaviour. On the one hand, one could suppose that each channel opened in a graded manner with voltage. According to this view, we might expect that, if the conductance of just one channel could be measured at different voltages, a single channel would show a conductance against voltage plot like that exhibited in Fig. 2. On the other hand, each channel might have only two conductance states, open and closed, and the gross conductance against voltage curve (Fig. 2) might simply reflect the fraction of channels open at any voltage. Of course, intermediate cases are easily envisaged. A channel might have, say, four different conductance levels (closed, 1/3 open, 2/3 open, fully open), and Fig. 2 would reflect the fraction of channels in each of their conductance states. Clearly, one cannot formulate a theory for channel opening mechanisms without knowing which of these possibilities is correct. Fluctuation analysis has been used to investigate this important question, and is, unfortunately, the only approach currently available.

At the acetylcholine-activated channels of the nerve-muscle junction increasing the dose of acetylcholine causes a larger conductance by causing more channels to open, but  $\gamma$  has the



**Fig. 2** A typical plot of steady-state conductance for one channel type (potassium, for example) as a function of voltage difference between the interior of a nerve cell and the exterior solution. The ordinate presents conductance as a fraction of the maximum possible value.

same value irrespective of how many channels are opened on the average. For the nerve membrane, channels open in response to the membrane voltage rather than the presence of a neurotransmitter like acetylcholine, but in this case as well,  $\gamma$  should be independent of the number of open channels if each channel has only two conductance states, open and closed. If, on the other hand, each channel has many conductance states, with the higher conductances favoured by positive voltages, one might expect that  $\gamma$  would increase as the total conductance were increased by subjecting the membrane to positive voltages. Although the actual situation turns out to be somewhat more complicated than these simplest expectations, fluctuation analysis can show whether  $\gamma$  remains constant as the total conductance increases.

The use of fluctuation analysis to get an indication of how many conductance states a type of channel might have is best introduced by considering the limiting case in which channels can be only open and closed. According to simple coin-flipping theory<sup>7,8</sup>, we know that the average conductance is

$$g = \gamma Nf \quad (2)$$

where  $\gamma$  is the single channel conductance,  $g$  is the average total membrane conductance,  $N$  is the number of channels in the membrane (they are assumed to be independent), and  $f$  is the probability that a channel is open. The quantity  $f$  would depend on voltage, and thus so would  $g$ . The variance in conductance is

$$s^2 = \gamma^2 Nf(1-f); \quad (3)$$

where  $s^2$  is the variance and other symbols have the same meaning as in equation (2). The quantities  $g$  and  $s^2$  can be measured in experiment, and  $f$  can be estimated from the conductance/voltage function such as appears in Fig. 2 if we assume that maximum conductance corresponds to a channel certainly being open ( $f$  is then just the fraction of the maximum conductance). Eliminating the unknown quantity  $N$  from equations (2) and (3), we can solve for  $\gamma$ , the single channel conductance, in terms of measurable quantities  $g$ ,  $s^2$ , and  $f$ :

$$\gamma = \frac{s^2}{g(1-f)} \quad (4)$$

Equation (4) gives a means of estimating single channel conductance if channels have only two conductance states.

If the excitable membrane channels have only two conductance states then  $\gamma$  estimated by equation (4) should, like the acetylcholine-activated channels described earlier, always have the same value. (Actually channels show rectification under some circumstances, so  $\gamma$  might appear non-constant. This rectification can be taken into account by measuring the instantaneous voltage-current relation and using the information to refer values of  $\gamma$  to some reference voltage.) As the membrane voltage is made more positive the probability that a channel is open approaches unity; the average number of open channels thus increases with positive voltages, so the average conductance  $g$  might increase along a sigmoid curve like that illustrated in Fig. 2. The variance in conductance fluctuations should, however, follow an inverted U-shaped function of voltage (according to equation (3)) that gives a constant  $\gamma$  estimate.

What would be expected if single channels have multiple conductance states so that the average conductance of a channel increases as the membrane voltage is made more positive? In general,  $\gamma$  as defined by equation (4) would not for this case be a constant independent of membrane voltage, but the exact behaviour of  $\gamma$  as the total conductance increased would depend on the exact mechanism underlying the conductance increase. Specifically,  $\gamma$  (according to equations (4)) might increase, decrease, or remain constant as  $g$  was increased by making the membrane voltage more positive. If the primary means by which the membrane increases its overall conductance is by making each channel more conductive,  $\gamma$  would, on the simplest theories, increase as  $g$  did. It is not difficult, however, to construct specific examples in which  $\gamma$  remains constant or decreases as  $g$  is increased.

In summary, measuring  $\gamma$  as a function of the mean conductance  $g$  can give an indication about whether individual channels have two or more than two conductance states. If a channel can only be open and closed,  $\gamma$  must be constant. If  $\gamma$  calculated from equation (4) is actually observed to be constant, the simplest interpretation is that channels can be only open or closed. The experiments performed so far indicate that  $\gamma$  is constant as  $g$  increases, so gates seem to behave in the simple open-closed way.

Three investigations of single channel conductance, estimated by equation (4), as a function of the total conductance  $g$  have appeared, all on frog nerve; one study was on potassium channels<sup>20</sup>, and two on sodium channels<sup>21,22</sup>. Begenisich and Stevens<sup>20</sup> could find no significant change in  $\gamma$  for K channels over a range of voltages that give from 15% to 85% of the maximum conductance. These workers found that the single channel conductance was 4 pS ( $4 \times 10^{-12}$  ohm<sup>-1</sup>), a value about 7 times lower than that for the acetylcholine-activated channels.

Sodium channels are more difficult to investigate than are potassium channels because of sodium channel inactivation: at most voltages, sodium channels open only transiently. The opening process is called activation and the closing, in the face of a maintained positive voltage, is known as inactivation. For example, if the membrane voltage is changed from a resting value of -60 mV to +40 mV, the sodium conductance would rapidly increase, and then decrease to nearly zero even though the voltage was kept constant. After becoming inactivated, sodium channels must have a rest period at a negative voltage before they can be activated again. This process of inactivation not only complicates the theoretical analysis of sodium channels, but also hinders the application of fluctuation analysis that requires observation to be made during a relatively long period during which the average conductance remains nearly constant. Potassium channels also show a similar inactivation phenomenon, but the rate of development of the inactivated state is so slow that sufficiently long samples with an insignificant change in mean conductance can be obtained.

Conti *et al.*<sup>22</sup> have studied fluctuations to investigate the behaviour of the sodium channel  $\gamma$  as the average conductance was varied. Because of the presence of sodium channel inactivation, however, these workers were restricted to a somewhat smaller range of voltages than were Begenisich and Stevens. As

with the potassium channel,  $\gamma$  remained constant over the entire accessible voltage range with a value of about 8 pS.

More recently Sigworth<sup>22</sup> has approached the problem with a different method that does not assume stationarity and that therefore circumvents the difficulties arising from the presence of sodium channel inactivation. The studies described until now have all carried out an analysis of fluctuations that occur over time around a constant or nearly constant mean value. Sigworth collected an ensemble of records with a transient rise and fall in sodium conductance. These records should all have been identical except for probabilistic fluctuations. After evaluating the source of variability other than from the random opening and closing of channels, Sigworth calculated the variance about the mean from all records at each time point. Equation (4) should still hold, except that the mean and variance are calculated for an ensemble of records rather than over time for a single record.

Using this approach, Sigworth was able to estimate  $\gamma$  over the entire range from the resting voltage to +150 mV. In confirmation of the findings of Conti *et al.*, these experiments give a constant  $\gamma$  with a value of 8 pS under normal conditions. The value of  $\gamma$  was unchanged by treatment with tetrodotoxin, agent that decreases the number of functional channels present, but was decreased by lowering pH. This pH effect is consistent with a blocking action of hydrogen ions on ionic conduction through the channel<sup>23</sup>.

Although these studies are certainly not conclusive, they do constitute the first experimental support for the notion that channels in the nerve membrane, like the acetylcholine-activated channels at the nerve-muscle junction, have only two main conductance states, open and closed. If sustained by future research, this conclusion greatly simplifies the understanding of gating mechanisms.

The first application, of fluctuation analysis to nerve membrane, thus relates to evidence for an all-or-none gate on potassium and sodium channels. Another use, and one that will probably be more important, is in distinguishing between various possible molecular mechanisms of gating. Even at a constant voltage, the time course by which nerve channels open and close is quite complicated and requires a fairly high order differential equation for a good description. The Hodgkin-Huxley equations for potassium channels, for example, involve a fourth order differential equation. The difficulty is that various possible underlying mechanisms can give rise to the same equation for average behaviour, and an even larger number of mechanisms is consistent with similar mathematical descriptions that would, in

practice, be indistinguishable in their predictions for average behaviour. In general, however, distinct molecular mechanisms that predict identical or very similar average behaviour can make quite different predictions about the spectra of conductance fluctuations. Fluctuation analysis, then, has the potential of helping to decide between various alternative mechanisms that equally well account for the average behaviour of nerve channels. This approach has already been successfully used by Ruff, as described earlier, in investigations of local anaesthetic actions.

A number of investigators have published spectra of conductance fluctuations for nerve membranes<sup>21,24-29</sup>. So far, however, for reasons that are not yet clear, no two laboratories have managed to agree on the form and amplitudes of the spectra they find. The nature of these disagreements has recently been detailed by Nether and Stevens<sup>6</sup> and will not be repeated here.

To summarise, one may say that fluctuation analysis has already helped in understanding gating mechanisms at the nerve-muscle junction as indicated by the examples given earlier. Further the technique has given the first experimental evidence that nerve channels gates behave in all all-or-none manner. The challenge for the future will be to make use of this approach to elucidate the processes underlying the phenomena described by the Hodgkin-Huxley equations.

1. Hodgkin, A. L., Huxley, A. F. & Katz, B. *J. Physiol., Lond.* **116**, 424-448 (1952).
2. Hodgkin, A. L. & Huxley, A. F. *J. Physiol., Lond.* **116**, 449-472 (1952); *ibid.* **116**, 473-496 (1952); *ibid.* **116**, 497-506 (1952); *ibid.* **117**, 500-544 (1952).
3. Stevens, C. F. *Fed. Proc.* **34**, 1364-1369 (1975).
4. Verveen, A. A. & DeFelice, L. J. *Prog. Biophys. molec. Biol.* **28**, 189-265 (1974).
5. Conti, F. & Wanke, E. *Q. Rev. Biophys.* **8**, 451-506 (1975).
6. Neher, E. & Stevens, C. F. *Ann. Rev. Biophys. Bioeng.* **6**, 345-381 (1977).
7. Feller, W. *An Introduction to Probability Theory and Its Applications* **2** (Wiley, New York, 1966).
8. Moran, P. A. P. *An Introduction to Probability Theory* (Clarendon, Oxford, 1968).
9. Magleby, K. L. & Stevens, C. F. *J. Physiol., Lond.* **223**, 151-171 (1972); *ibid.* **223**, 173-197 (1972).
10. Katz, B. & Miledi, R. *Nature* **226**, 962-963 (1970); **224**, 665-699 (1972).
11. Anderson, C. R. & Stevens, C. F. *J. Physiol., Lond.* **235**, 655-691 (1973).
12. Neher, E. & Sakmann, B. *Nature* **260**, 779-802 (1976).
13. Colquhoun, D., Large, W. A. & Rang, H. P. *J. Physiol., Lond.* **266**, 361-395 (1977).
14. Katz, B. & Miledi, R. *J. Physiol., Lond.* **230**, 707-717 (1973).
15. Ruff, R. L. *Biophys. J.* **16**, 433-439 (1976); *J. Physiol.* **264**, 89-124 (1977).
16. Derksen, H. E. & Verveen, A. A. *Science* **151**, 1388-1389 (1966).
17. Verveen, A. A. *Fluctuation in Excitability* 1-76 (Drukkerij Holland N.V., Amsterdam, 1961).
18. Blair, E. A. & Erlanger, J. *Am. J. Physiol.* **106**, 524-564 (1933).
19. Pecher, C. *Arch. Internat. Physiol.* **49**, 129-152 (1939).
20. Begeisch, T. & Stevens, C. F. *Biophys. J.* **15**, 843-846 (1975).
21. Conti, F., Hille, B., Neumcke, B., Nonner, W. & Stämpfli, R. *J. Physiol., Lond.* **262**, 699-727 (1976); *ibid.* **262**, 729-742 (1976).
22. Sigworth, F. J. *Nature* **270**, 265-267 (1977).
23. Woodhull, A. M. *J. gen. Physiol.* **61**, 687-708 (1973).
24. Siebenga, E., Meyer, A. W. A. & Verveen, A. A. *Pflügers Arch.* **341**, 87-96 (1973).
25. Fishman, H. M. *Proc. nat. Acad. Sci. U.S.A.* **70**, 876-879 (1973).
26. Fishman, H. M., Moore, L. E. & Poussart, D. J. *M. J. membr. Biol.* **24**, 305-328 (1975).
27. Conti, F., DeFelice, L. J. & Wanke, E. *J. Physiol., Lond.* **248**, 45-82 (1975).
28. Fishman, H. M. *J. membr. Biol.* **24**, 265-277 (1975).
29. van den Berg, R. J., de Goede, J. & Verveen, A. A. *Pflügers Arch.* **360**, 17-23 (1975).

# articles

## Hubble's constant determined from super-luminal radio sources

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*Recent data on the so-called super-light expansion velocities observed in the radio galaxy 3C120 show a good fit to the light echo theory, provided the Hubble constant is  $110 \pm 10 \text{ km s}^{-1} \text{ Mpc}^{-1}$ . Statistics on three super-luminal sources interpreted with that theory give the same Hubble constant. A method of determining  $q_0$  from each source with data that may already exist, looks very promising.*

REES and others<sup>1,2</sup> have displayed remarkable ingenuity in devising mechanisms that can lead to high multiples of  $c$  in apparent transverse motion, nevertheless, the only theory that looks plausible for general application is the old 'light echo' theory<sup>3</sup>. In 1901 Nova Persei exploded close to a reflection nebula and its light echo was seen on the nebula with an apparent transverse expansion of a diameter of  $2c$ , and was used to determine a distance to Nova Persei<sup>4</sup>. I aim to show here that the

mystery that surrounds the super-luminal velocities observed in radio sources is a myth, and that all well observed objects can be explained with this old theory. The mystery has developed because most authors preferred too low a value of the Hubble constant and the interpretation of the data has caused some confusion. This investigation was stimulated by the account of Cohen *et al.*<sup>5</sup> of both super-luminal velocities and the ambiguities of their interpretation.

My accretion disk picture<sup>6</sup> of quasars and Seyfert galaxies<sup>7-10</sup> has the inner part of the disk inflated into a nearly spherical shape by radiation pressure<sup>10,11</sup>. Just as water flowing into the black hole in a bath does so around a narrow vortex, so the sphere is pierced by a narrow vortex hole on the sides of which velocities close to  $c$  are reached as the central black hole is approached. X rays, strong electromagnetic waves and relativistic plasma generated close to the hole can only escape up or down the narrow tube formed by this vortex. I shall assume that brilliant flashes of radiation (or alternatively relativistic shock waves moving with velocity  $c$ ) emerge up these oppositely-directed holes and that their energy density is so great that they generate relativistic random motions in the plasma they illuminate. This causes the plasma to radiate by the synchrotron process. The patches illuminated by a flash from the centre each move out with the velocity of light. If the tube were perpendicular to the line of sight the apparent transverse expansion apart of the two components would be  $2c$ . Allowance for an angle  $\theta$  between the tube and the observers's line of sight, however, changes this to  $2c/\sin \theta$ .

There are two immediate objections to this theory. (1) 3C120 has shown two flashes at  $5c$  and  $8c$  so that  $\theta$  must change over a short period, but the position angle on the sky remains unchanged. I shall show that the second expansion velocity is caused by a misinterpretation and that the events in this galaxy fit the theory perfectly. (2) There are too many very large multiples of  $c$  observed in apparent expansions. This is explained by the change of Hubble constant together with some over-concentration on more esoteric interpretations of observed events. In particular Cohen *et al.*<sup>5</sup> point out that events in 3C279 can either be interpreted as a  $10c$  expansion ( $5c$  on the new Hubble constant) or as three fixed sources of variable intensity. No doubt two moving sources and an off-central source of variability would be as good.

The light echo theory used here suggests rules of interpretation which should help to build much better models from very long baseline (VLB) data. These in turn will yield values for  $q_0$  the deceleration parameter that determines the geometry of the universe. Data probably already exist which will allow this to be done for 3C345, 3C279, 3C454.3.

Here a simple account of the light echo theory and its predictions is given. These are used to determine the Hubble constant in two different ways. First, from the data on 3C120 and second, from the statistics of the three well-observed super-luminal expansions.

### Simple light echo theory for non-cosmological distances

Consider an object O not moving with respect to the radio antenna A. Let its tube point at  $\theta$  to the line of sight. Then a flash from O will illuminate and excite material at a source  $S_1$  distant  $ct$  from it. This event will be seen by A not at time  $t$  after he sees the initial burst at O but at time  $t - (ct \cos \theta)/c$  because the source  $S_1$  is nearer than O. The observed transverse velocity of  $S_1$  is

$$[ct \sin \theta]/[t(1 - \cos \theta)].$$

That is

$$v_1/c = \sin \theta / (1 - \cos \theta) \quad (1)$$

The source flying in the opposite direction has apparent transverse motion  $v_2$  where

$$v_2/c = \sin \theta / (1 + \cos \theta) \quad (2)$$

The apparent separation velocity is the sum of these

$$v_s = 2c \sin \theta / (1 - \cos^2 \theta) = 2c / \sin \theta \quad (3)$$

Note that the apparent velocity of separation can not be  $< 2c$ . Also if  $S_1$ ,  $S_2$  and O are all seen, then the separation ratio

$$\frac{S_1 O}{S_2 O} = \frac{1 + \cos \theta}{1 - \cos \theta} \quad (4)$$

and so  $\theta$  can be determined direct from such observations. With  $\theta$  known and the apparent angular separation rate  $\dot{\chi} = v_s/D$  found from VLB observation then the distance  $D$  is  $v_s/\dot{\chi} = 2c(\dot{\chi} \sin \theta)^{-1}$ . The Hubble constant is then determined from the redshift  $z$  of the object at O by  $H_0 = cz/D$ .

### Modification factors

The above theory is subject to simple modification when the object O is not observed at rest but nearby at a large redshift  $z$ . In the first case the observer allowing for time dilation attributes an 'apparent' expansion velocity  $v_s$  related to  $\dot{\chi}$  by  $\dot{\chi} = v_s/[D(1+z)]$ . The events seem to happen slower than they really do because of the redshift. Second, the great time lapse between the emission and the reception of the radiation is large and the geometry may not be Euclidean or Minkowskian. In particular in Friedmann universes the formula connecting the apparent transverse motion  $\dot{\chi}$  and  $v_s$  is not

$$v_s = D\dot{\chi}(1+z) \doteq \frac{cz}{H_0}(1+z)\dot{\chi} \quad (5)$$

where  $H_0$  is the Hubble constant, but is modified by factors dependent on the acceleration parameter  $q_0$ . The full formula (given in ref. 5) is

$$\frac{v_s}{c} = \frac{\dot{\chi}}{H_0} q_0^{-2} (1+z)^{-1} \{q_0 z - (1-q_0)[(1+2q_0 z)^{1/2} - 1]\} \quad (6)$$

for  $q_0 z \ll \frac{1}{2}$  this gives

$$\frac{v_s}{c} = \frac{z}{H_0} (1+z)^{-1} [1 + \frac{1}{2}(1-q_0)z] \quad (7)$$

Note the correction factor of  $(1+z)^{-2} [1 + \frac{1}{2}(1-q_0)z]$  as compared with the simple equation (5). Equation (4) being a ratio of separations still holds good as do equations for  $v_1/v_s$  and  $v_2/v_s$  obtained by dividing equation (1) by (3) and equation (2) by (3).

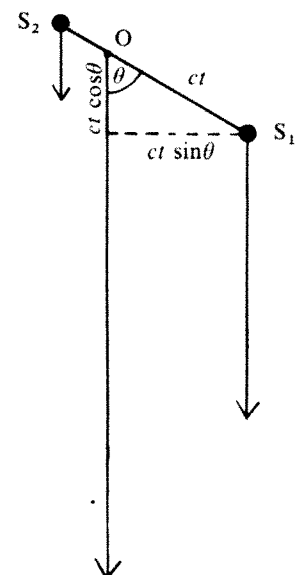


Fig. 1 A flash at O in a tube illuminates and excites two patches of plasma seen by the antenna A at positions  $S_1$  and  $S_2$ . Whereas both  $S_1$  and  $S_2$  actually travel out at the speed of light,  $S_2$  is seen at an earlier source epoch because it is further away.



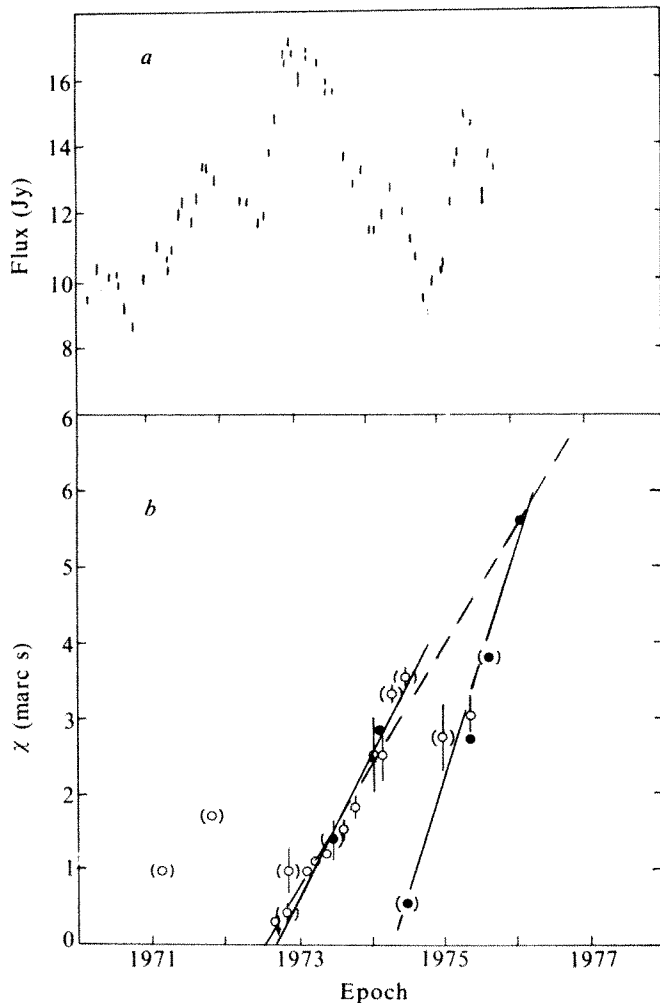


Fig. 2 The observations of 3C120 as reported in ref. 5 but with a dotted line superposed. *b*, plots  $\chi$  the separation angle  $S_1AS_2$  against time.  $\bullet$ , 2.8 cm;  $\circ$ , 3.8 cm;  $\blacktriangle$ , 6.0 cm. *a*, The 3.8 cm flux.

### Application to 3C120

Figure 2 shows the observations given in ref. (5). The 3.8-cm flux shows an outburst in 1973 which caused a double source. This was seen as a simple double expanding at about  $v_s = 5c$  (solid line) until 1974.2 when the double model failed to fit well, because of a small increase of flux. In 1975 there followed a violent increase of flux and immediately a two source model fitted well again, but at about the same separation as it was at 1974.0. The source began to dim in late 1975 and in 1976.1 it was again a well-determined double although the flux then was not given. Cohen *et al.* interpreted these events as the two super-luminal expansions of  $5c$  and  $8c$  given by the heavy straight lines. In my interpretation, however, this would involve the vortex tube up the rotation axis of the radio galaxy to turn between the two events and I consider this unlikely, especially as the observed position angle of separation on the sky are the same for both doubles. The following interpretation of events is more natural. 1972.5–1973 a double expanding source  $S_1, S_2$  was born at O and expanded throughout 1973 as its flux diminished. In early 1974, the object at O brightens, confusing the neat double interpretation of the data. In 1975.2, O has increased in brightness so much that it forms a double with  $S_1$ , the source moving towards the Earth and  $S_2$  is outshone and ignored. O dims in late 1975 leading to a confused picture with  $S_1, O$  and  $S_2$  all seen. Finally in 1976, O dies away leaving the old expanding source  $S_1 S_2$ . This latest point fits on the dotted expansion line ( $4.6c$ ) of the old source, which is a good alternative to the full line ( $5c$ ) if one ignores confused data. The importance of this new interpretation arises because it not only removes the discrepancy of two different super-luminal velocities observed from the same object, and therefore probably at the same  $\theta$  but, because we see O at times we can

measure the ratio of the separations  $OS_1$  to  $OS_2$  and hence determine  $\theta$ . Hence, the apparent transverse separation velocity is known from equation (3) so the observed angular velocity gives the distance and hence Hubble's constant. The small  $z = 0.033$  of 3C120 makes the cosmological corrections unimportant for this source, so the Hubble constant derived in this case is independent of  $q_0$ . The same method applied to 3C345 should also yield a good value of  $q_0$ .

If  $\theta$  were  $90^\circ$  the clear double seen in 1975.4 would have been half way up to the dotted line. Its fractional offset  $[OS_1 - \frac{1}{2}S_1S_2]/S_1S_2$  corresponds to the offset of O from the centre of  $S_1$  and  $S_2$  and is, therefore,  $\frac{1}{2} \cos \theta$ , from this  $\theta = 68^\circ \pm 10^\circ$ . It is particularly interesting that this angle is close to the inclination of the apparent disk of 3C120 measured from Arp's picture on which it seems to be a disturbed spiral<sup>12</sup>. From his picture,  $i = 55\text{--}60^\circ$  but this may be in error by  $10^\circ$  due to the fact that the disturbed disk does not have a circular outline. Furthermore the observed separation of the radio double, although fairly close to the apparent minor axis of the optical image is not along it. It seems reasonable to assume that ejection is taking place along the rotation axis, but the galaxy has imperfect circular symmetry. In such a case  $i$  should equal  $\theta$  and as both determinations have similar errors we shall take  $\theta = 64 \pm 8^\circ$ . The light echo theory then gives

$$v_s = 2c/\sin 64^\circ = 2.22c \pm 0.19c.$$

Cohen *et al.* adopt  $H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$  and obtained  $5c$  for this velocity, but our correction to the dotted line interpretation of Fig. 2 yields  $4.6c$  on that scale. To deduce the answer from the light echo theory the Hubble constant must be changed by  $(4.6 \pm 0.2)/(2.22 \pm 0.19) = 2.1 \pm 0.2$  producing Hubble constant of  $115 \pm 12 \text{ km s}^{-1} \text{ Mpc}^{-1}$ .

### Application to 3C273

Triple models have been given<sup>13</sup> for 3C273 and the best ratio of source distances from the centre gives  $\cos \theta = 0.2 \pm 0.2$ ,  $\theta = 78.5^\circ \pm 12^\circ$ . The expected expansion is  $2c/\sin \theta = 2.04c$  while the observations give  $4.2c$  on the  $H_0 = 55$  scale, with  $q_0 = 0.05$ . The correction factor is thus  $4.2/2.04 = 2.05$  yielding a Hubble constant of  $113^{+3}_{-10} \text{ km s}^{-1} \text{ Mpc}^{-1}$ .

### Statistics of super-luminal expansions

From equation (3)  $v_s = 2c/\sin \theta$  so this velocity cannot be less than  $2c$ . Furthermore most of the solid angle of a sphere is not too far from its equator, so  $v_s$  ought often to be just greater than  $2c$ , whereas  $v_s > 6c$  should occur only seldom. The times a super-luminal velocity less than some chosen  $v_s$  is seen should be

$$\frac{N(v_s)}{N_0} = (2\pi)^{-1}[(2\pi - 2\pi(1 - \cos \theta))] = \cos \theta = \left[1 - \left(\frac{2c}{v_s}\right)^2\right]^{1/2} \quad (8)$$

for  $v_s > 2c$  or for  $v_s < 2c$

The distribution function for  $v_s$  should be  $-d(N/N_0)/dv_s$  and this is plotted as Fig. 3. With only three well determined super-luminal velocities to consider, however, it is better to consider the formulae for the integrated distribution and its moments. In particular averaged over angle

$$\left\langle \frac{1}{v_s} \right\rangle = \int \frac{1}{v_s} \frac{dN}{N_0} = \frac{\pi}{8c} = \frac{0.79}{2c} \quad (9)$$

and

$$\sigma^2 = \left\langle \left( \frac{1}{v_s} - \left\langle \frac{1}{v_s} \right\rangle \right)^2 \right\rangle = \left( \frac{2}{3} - \frac{\pi^2}{16} \right) \frac{1}{4c^2} \quad (10)$$

$$\sigma = 0.22/2c.$$

The three well determined super-luminal velocities are, on the  $H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$  scale with  $q_0$  assumed small ( $q_0 = 0.05$ ),  $v_s/c = 4.6, 4.2$ , and  $7.0$  in the objects 3C120, 3C273 and 3C345 respectively. Thus with standard errors for the mean

$$\left\langle \frac{1}{v_s} \right\rangle = (0.20 \pm 0.05) \frac{1}{c} = (0.40 \pm 0.10) \frac{1}{2c}$$

To scale this to the theoretical value of equation (9) we must change

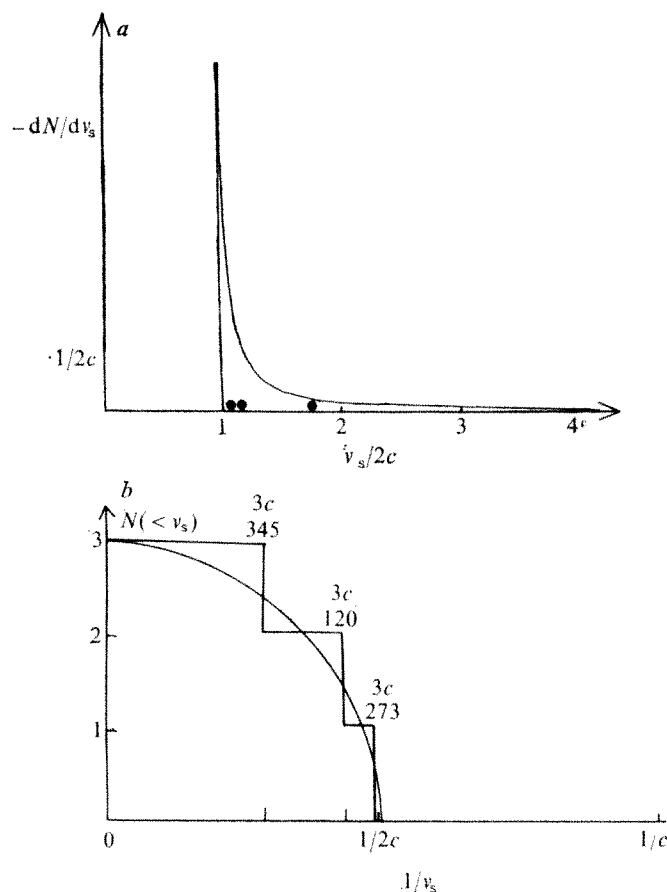


Fig. 3 a, The distribution of superluminal velocities expected from the light echo theory, and b, its integral plotted against  $2c/v_s$  compared with the histogram of observed data.

Hubble's constant by  $0.79/(0.4 \pm 0.1) = 2.0 \pm 0.5$  giving  $H_0 = 110 \pm 30$ . One further constraint on the factor may be independently deduced from the expansion of 3C273 at  $4.2c$  on the  $H_0 = 55$  scale. From our theory this must be greater than  $2c$  so the correction factor cannot be larger than 2.1. Taking this and its error into account the best value of the correction factor is  $2.0 \pm 0.1$  and the Hubble constant is  $110 \text{ km s}^{-1} \text{ Mpc}^{-1}$  with a maximum error upwards of  $10 \text{ km s}^{-1} \text{ Mpc}^{-1}$  and an r.m.s. error downwards of about 10 also.

Principles for interpreting super-luminal source data on light

echo theory are (1) Bursts occur at the source O. (2) They usually cause pairs of expanding sources  $S_1, S_2$  of which  $S_1$ , the one nearer the observer, is normally seen as the brighter. Since  $S_2$  is seen at an earlier source epoch it may have a flatter (harder) spectrum than  $S_1$ . (3)  $S_1$  and  $S_2$  expand outwards at velocities  $c \sin \theta / [1 - \cos \theta]$  and  $c \sin \theta / [1 + \cos \theta]$  respectively. These velocities do not accelerate. (4) Further bursts may occur at O leading to a brightening of O possibly followed by further source pairs  $S_1', S_2'$  travelling along the same position angle and at the same velocities as  $S_1$  and  $S_2$  respectively. (5) All source pairs will eventually fade but the source O is responsible for all very rapid time variability.

### Predictions

The light echo interpretation of the 3C120 data suggest that when the 3.8 cm flux at 1976.1 is disclosed it will again be low. Also that the next experiment will show a point on the dotted line of Fig. 2 if the flux continues low. It also suggests that the ratio  $S_2O/S_1O$  will be about 0.10 if O is seen in 3C345 (we may already have seen O and not  $S_2$  in which case we still expect the same ratio).

The new value of  $H_0$  predicts that no super-light velocities  $v_s$  less than  $4c$  on the old  $H_0 = 55$  scale will be detected. If, however,  $S_2$  were not seen the separation speeds of  $v_1$  down to  $2c$  on that old scale could be found. Finally each source should give the same value for  $H_0$  if this interpretation is correct.

### Conclusions

The Hubble constant is  $110 \pm 10 \text{ km s}^{-1} \text{ Mpc}^{-1}$ . The universe is half the linear size and half the age that would be attributed to it on the old Hubble constant of  $55 \text{ km s}^{-1} \text{ Mpc}^{-1}$ . The new  $H_0^{-1}$  age is  $9 \times 10^9$  yr and this means that Einstein-de Sitter age of  $\frac{2}{3}H_0^{-1}$  is embarrassingly small for stellar evolution theory. Hence, it is probable that the Universe is open with a small value of  $q_0$ . For many years Van den Bergh and de Vaucouleurs, and more recently Dibai, Hanes, Hartwick and Madore, have advocated almost as large values of the Hubble constant.

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1. Rees, M. J. *Nature* **211**, 468 (1966).
2. Blandford, R. D., McKee, C. F. & Rees, M. J. *Nature* **267**, 211 (1977).
3. Perrine, C. D. *Astrophys. J.* **17**, 310 (1903).
4. Russell, H. N., Dugan, R. S. & Stewart, J. Q. *Astronomy* **2**, 786 (Ginn & Co., Boston, 1927).
5. Cohen, M. H. *et al.* *Nature* **259**, 17-20 (1976).
6. Lynden-Bell, D. *Proc. Copenhagen Quasar Conf.* (1977).
7. Lynden-Bell, D. *Nature* **223**, 690 (1969).
8. Lynden-Bell, D. *Vatican Symp. Nuclei of Galaxies* 527 (ed. O'Connell, D.) (Pont. Acad. Sci., 1970).
9. Lynden-Bell, D. & Rees, M. J. *Mon. Not. R. astr. Soc.* **175**, 613 (1971).
10. Bardeen, J. M. *Nature* **226**, 64 (1970).
11. Shakura, N. I. & Sunyaev, R. A. *Mon. Not. R. astr. Soc.* **175**, 613 (1976).
12. Arp, H. *Pub. astr. Soc. Pac.* **87**, 546 (1976).
13. Schilizzi, R. T. *et al.* *Astrophys. J.* **201**, 263 (1975).

## Optimum efficiency of photogalvanic cells for solar energy conversion

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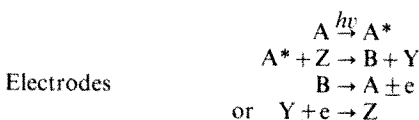
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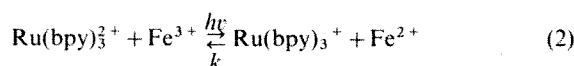
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The performance of photogalvanic cells for the direct conversion of solar energy to electrical energy depends on the cell photochemistry, the homogeneous kinetics, the mass transport, the electrode kinetics and the load on the cell. The variation of the power output with the concentrations of the redox couples, their transport and kinetic parameters and the dimensions of the cell is found. The power conversion efficiency of the optimal cell could be as large as 18% but it is unlikely that all the necessary conditions can be met. A more realistic estimate of the maximum power conversion efficiency that could be achieved from a photogalvanic cell is between 5 and 9%.

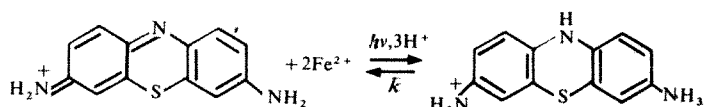
PHOTO GALVANIC cells are possible devices for the direct conversion of solar energy into electrical energy. This paper is concerned with the maximum power that can be achieved by such devices and with describing the conditions for the most efficient operation of such cells. The cell works by the reactions



Two redox couples A,B and Y,Z are present in the solution; in the dark there is a very small concentration of B. On illumination, the absorption of radiation by A and the subsequent electron transfer reaction of A\* with Z generates B. The photogenerated B reacts on one of the electrodes thereby generating power. Examples of this type of system are shown in equations (2) and (3). We assume that the concentrations of Y and Z are large, compared to the concentration of A so that the concentrations of Y and Z throughout the cell are not significantly perturbed from their values in the dark.



where bpy = 2,2'-bipyridine.



The best geometry for the cell consists of two parallel electrodes separated by a thin layer ( $\sim 0.1$  mm) of electrolyte solution. One of the electrodes is transparent (such as  $\text{SnO}_2$ ); the light is shone through this electrode, as shown in Fig. 1.

### Reactions on electrodes

The performance of the cell depends first on the kinetics of the electrode reactions. On either electrode, either couple can have 'reversible' or 'irreversible' electrode kinetics. When a couple is reversible the electrode kinetics are rapid and the surface concentrations of the couple at the electrode obey the Nernst equation. When a couple is irreversible the electrode kinetics are sluggish and we only consider the extreme case where no electrode reaction takes place. The performance of cells, in which couples have intermediate electrode kinetics, will be between the two extremes that we consider. Four of the 16 possible cases are shown in Table 1.

Eight cases which have irreversible electrode kinetics for both couples on either of the electrodes can be rejected because to pass current it is essential that one of the couples should be reversible on each electrode. Four cases in which the Y,Z couple is reversible on both electrodes can be rejected because we have assumed that there is an insignificant perturbation to the concentrations of the Y,Z couple; so if the couple is reversible, the two electrodes will have the same potential and no power is produced. Similarly the fourth case of Table 1 can be rejected because at the dark electrode the reversible A,B couple will give the same potential as the reversible Y,Z couple at the illuminated electrode. This leaves three cases worth considering. In the first, the electrode kinetics are similar on the two electrodes and hence they can be made of the same material. The cell works as a 'concentration cell'. In the

other two cases the electrode kinetics are different on the two electrodes and the cell works with 'differential electrode kinetics'.

### Concentration of B in solution

To find the power developed by the cell we have to calculate how the concentration of B varies with the distance between the two electrodes. A steady state is established described by the differential equation (4)

$$D \frac{\partial^2 b}{\partial x^2} + \phi I \epsilon a - kby = 0 \quad (4)$$

where  $D$  is the diffusion coefficient of B;  $x$  describes the distance from the illuminated electrode;  $\phi$  is the quantum efficiency for the generation of B from A;  $I$  is the quantum intensity of the light;  $\epsilon$  is the natural extinction coefficient of A;  $a$ ,  $b$ , and  $y$  are the concentrations of A, B and Y respectively; and  $a_0$  is the concentration of A in the unilluminated solution.

The first term describes the diffusion of B, the second term its photochemical generation and the third term the thermal reaction with Y. There is no term describing transport by convection because we assume that the electrodes are close enough ( $< 0.1$  mm) for natural convection not to develop. There is no advantage in having the electrodes further apart and there may be the disadvantage that the internal resistance of the cell may be larger.

The light intensity in equation (4) varies with  $x$  and obeys

$$\frac{\partial I}{\partial x} = -\epsilon a I = -\epsilon(a_0 - b)I \quad (5)$$

where we have assumed that the diffusion coefficients of A and B are equal so that  $a + b = a_0$ . This differential equation gives the Beer-Lambert law when  $b \ll a_0$ , but we have also investigated the situation where  $b \approx a_0$  which means that the solution is bleached.

The boundary condition for equation (5) is that at  $x = 0$ ,  $I = I_0$ .

The boundary conditions for equation (4) depend on the electrode kinetics. At the illuminated electrode for the first three cases in Table 1

$$\left( \frac{\partial b}{\partial x} \right)_0 = b'_0 = \frac{|i|}{AFD} \quad (6)$$

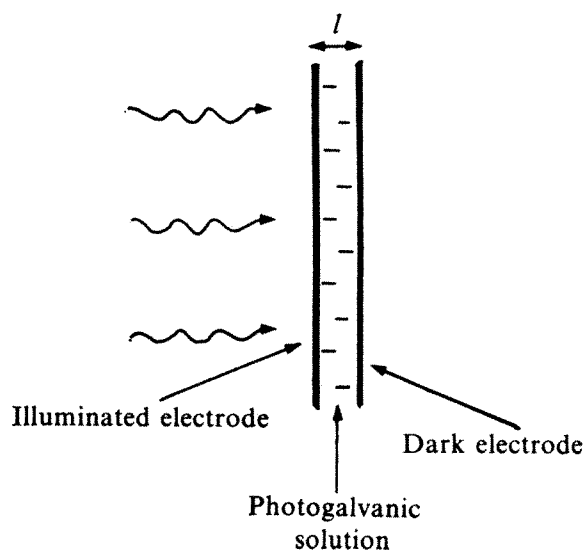
At the dark electrode the boundary conditions depend on the different electrode kinetics shown in Table 1 and are: concentration cell,  $(\partial b / \partial x)_1 = b'_0$ ; differential electrode kinetics case I  $(\partial b / \partial x)_1 = 0$ ; case II  $b_1 \rightarrow 0$ .

The case II boundary condition arises because with both couples reversible, the dark electrode re-establishes at its surface

Table 1 Reversible (R) or irreversible (I) electrode kinetics

	Illuminated electrode		Dark electrode		Notes
	A,B	Y,Z	A,B	Y,Z	
Couple					
Case 1	R	I	R	I	Concentration cell
Case 2	R	I	I	R	Differential electrode kinetics I
Case 3	R	I	R	R	Differential electrode kinetics II
Case 4	R	R	R	I	Reject

Fig. 1 Typical photogalvanic cell.



the equilibrium values of the concentrations found in the un-illuminated solution; any B is destroyed by the electrode:



The solution of the differential equations with the boundary conditions has been described elsewhere<sup>1,2</sup>. The value of  $b$  at the illuminated electrode allows the potential at that electrode to be calculated from the Nernst equation, as in all three cases the A,B couple is reversible. For the concentration cell a similar calculation is carried out at the dark electrode. For cells with differential electrode kinetics the potential difference at the dark electrode is unchanged from its value where the cell is un-illuminated. Hence for each value of the current using equation (6) we can calculate the potential difference across the cell and hence the power produced.

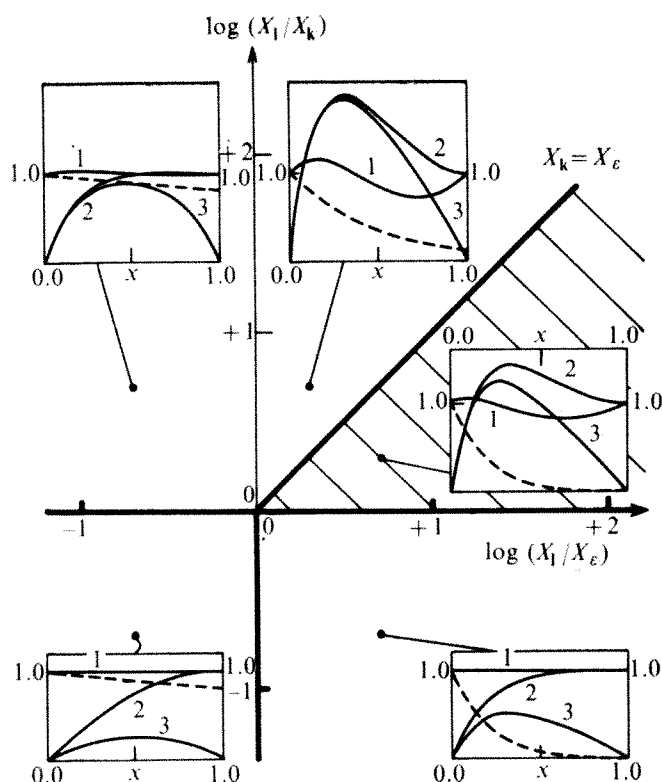
## Results

In giving the results of the calculations it is convenient to introduce the characteristic lengths given in Table 2. In Fig. 2 we show some typical concentration profiles for different values of the characteristic lengths and for the different electrode kinetics given in Table 1. The profiles are drawn for the case where the cell is short circuited. We also show how the light is absorbed in the cell. In all these cases the solution is not bleached and the generating length is unimportant. The current at short circuit is a maximum if the following condition holds:

$$X_e < X_k + X_l \quad (8)$$

The photoelectrochemical collection efficiency,  $N_{hv}$  (ref. 3) describes the flux of electrons from the cell divided by the incident flux of photons. For the conditions given by equation (8) it is equal to its maximum value of unity for a cell with differential electrode kinetics, (cases 2 and 3 of Table 1). All the photons are trapped close to the illuminated electrode and turned into current. For the concentration cell the maximum value of  $N_{hv}$  is  $\frac{1}{2}$  (S. W. Feldberg, personal communication). Even though the photons are trapped close to the illuminated electrode, the fact that the Y,Z couple is not electroactive on the dark electrode means that at short circuit the concentration of B has to be equal at the two electrodes. This in turn means that the photogenerated flux of B partitions equally between diffusion away from the illuminated electrode and diffusion away from the dark electrode after reaction at both electrodes.

Although the condition given in equation (8) maximises the current developed by the cell, the power also depends on the voltage at which the current is produced. The maximum power is found for cells in the hatched region of Fig. 2. For concentration cells it is essential to have the cell length,  $X_l$ , greater than the reaction length,  $X_k$ , or else B can diffuse all over the cell producing a uniform concentration profile and hence very little power. For the cells with differential electrode kinetics, increasing the concentration of Y increases the potential difference developed by the cell through the effect of Y on the potential of the Y,Z half cell at the dark electrode. But increasing the concentration of Y also increases the rate of the back reaction  $B + Y$  and hence decreases



**Fig. 2** Typical concentration profiles across photogalvanic cells. The illuminated electrode is on the left and the broken lines show the penetration of the light; the abscissa compares  $X_l$ , the cell length, with  $X_e$ , the absorbance length. The ordinate compares  $X_l$  with  $X_k$ , the reaction length, which describes the decomposition of the photo-generated intermediate B. The solid lines show the concentration of B for the first three cases in Table 1, when the cell is short circuited. In all three cases B reacts on the illuminated electrode. On the dark electrode B is generated, does not react and is destroyed for cases 1, 2 and 3 respectively. The cells are most efficient in the hatched region.

$X_k$ . Thus the optimum power point for this type of cell depends on the concentration of Y and is found in the hatched area close to the line  $X_k = X_e$ . Detailed investigation of the cases where the dye, A, is bleached shows that they produce less power than cases in the hatched region of Fig. 2. The voltage developed by such cells is larger but the current is very much less because the photons are absorbed too far away from the illuminated electrode for the photogenerated B to reach it before decomposing.

The maximum power  $W_{max}$  produced by the concentration cell<sup>1</sup> can be shown to be

$$W_{max} = 0.28 ART\phi I_0 \quad (9)$$

where  $A$  is the area of each electrode.

This is the maximum possible power that can be obtained after optimising the concentrations of the species, the dimensions of the cell and the load on the cell. The conditions for this maximum are:

$$X_e < X_G < X_k < X_l \quad (10)$$

and

$$X_G^3 = X_k^2 X_e \quad (11)$$

The maximum power produced by a cell with differential electrode kinetics can be shown to be<sup>2</sup>:

$$W_{max} = 0.8AF\phi I_0[|\Delta E| + \frac{RT}{F}\{\ln \frac{\phi I_0 e}{k[Z]} - 1.6\}] \quad (12)$$

$$\approx 0.8AF\phi I_0 \Delta E \quad (13)$$

where  $|\Delta E|$  is the difference in standard electrode potentials for the A,B and Y,Z couples.

**Table 2** Characteristic lengths

Name	Symbol and equation	Description
Cell length	$X_l$	Distance between electrodes
Absorbance length	$X_e = (\epsilon a_0)^{-1}$	Unless solution is bleached, the light is absorbed mainly in this distance
Generating length	$X_G = (D/\phi \epsilon I_0)^{1/2}$	Average distance A diffuses in light of intensity $I_0$ before being converted into B
Reaction length	$X_k = (D/k_Y)^{1/2}$	Average distance B diffuses before being converted to A



The conditions for this maximum are:

$$10 X_e \approx X_G \approx \frac{1}{2} X_k < X_l \quad (14)$$

If these conditions are fulfilled then equation (13) describes the maximum power from cells with differential electrode kinetics whether they be case 2 or 3 of Table 1.

## Discussion

For both types of cell we find the same order for the characteristic lengths in equations (10) and (14). The shortest length must be  $X_e$  to absorb all the photons close to the electrode. The inequality  $X_k > X_e$  ensures that the B, that is formed within the distance  $X_e$ , is not decomposed by reaction with Y before it reaches the electrode. The conditions for  $X_G$  ensure that the solution does not become bleached near the illuminated electrode. The condition for  $X_l$ , the distance between the electrodes, means that the dark electrode does not interfere with the trapping of the photons and the reaction of B on the illuminated electrode.

The maximum power that the concentration cell can deliver is given by equation (9). For the maximum power point the current is close to its short circuit value; the collection efficiency is  $0.3\phi$ , and the current density is  $0.3 \phi I_0$ . But the cell voltage is only about  $(RT/F) \ln$  term in the Nernst equation.

One cannot draw significant currents from a concentration cell and at the same time maintain very different concentrations at the two electrodes.

To calculate power outputs from equations (9) and (13) we must select a reasonable value for  $I_0$ . In AM2 solar radiation (of total irradiance  $749 \text{ W m}^{-2}$ ),  $I_0$  values for photons of wavelengths less than 700, 600 and 500 nm are  $1.57 \times 10^{-3}$ ,  $9.46 \times 10^{-4}$  and  $4.2 \times 10^{-4} \text{ mol photons m}^{-2} \text{ s}^{-1}$  respectively<sup>4</sup>. We shall take a value of  $1.6 \times 10^{-3} \text{ mol photons m}^{-2} \text{ s}^{-1}$  as a reasonable estimate of the flux density of photochemically active photons in AM2 sunlight. As relative values of solar spectral irradiance do not vary very greatly with atmospheric conditions<sup>5</sup>, conversion efficiencies calculated from these figures do not vary by more than a few per cent from efficiencies calculated from other solar spectral distributions; absolute outputs, of course, vary with the incoming total irradiance.

Taking  $I_0 = 1.6 \times 10^{-3} \text{ mol photons m}^{-2} \text{ s}^{-1}$  and  $\phi = 1$  we obtain from equation (9)

$$W_{\max}/A = 1.1 \text{ W m}^{-2} \quad (15)$$

This is the maximum power that can be obtained from a photogalvanic concentration cell driven by solar energy. The power conversion efficiency is no more than 0.15% and we conclude that the concentration cell cannot be an effective device for solar energy conversion.

For a cell with differential electrode kinetics, the maximum power is given by equation (13). Not only is the collection efficiency much closer to unity but also the cell voltage is now given by  $\Delta E$  rather than the miserable  $RT/F$  of the concentration cell. The optimum value for  $\Delta E$  is 1.1 V<sup>6</sup>. With this value and the same value of  $I_0$  as above we obtain

$$W_{\max}/A = 140 \text{ W m}^{-2} \quad (16)$$

This corresponds to a power conversion efficiency of 18%. In principle the optimum case for this type of cell is almost as efficient as a silicon solar cell. Nearly all the photons that can drive the reaction are trapped. While in the ideal case negligible energy would be wasted between the absorption of a photon with threshold energy and the formation of B, in the case we have taken the threshold photon energy is 700 nm or 1.8 eV and the flux of electrons is produced at the lesser potential difference of 1.1 V. Had we taken threshold photon energies of 600 or 500 nm the conversion efficiencies would have been 11% and 5% respectively. To achieve this amount of power we have to satisfy equation (14),

however. For typical values of  $D = 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  and  $\epsilon = 10^8 \text{ cm}^2 \text{ mol}^{-1}$  we find

$$X_G \sim 10^{-3} \text{ cm} \quad (17)$$

This means that

$$X_e \sim 10^{-4} \text{ cm} \quad (18)$$

or

$$[A] \sim 10^{-1} \text{ M} \quad (19)$$

Hence the dye, A, has to be rather soluble. Furthermore, the electrochemical rate constant  $k'$ , for the destruction of B on the illuminated electrode must satisfy the condition

$$k' > D/X_e \sim 10^{-1} \text{ cm s}^{-1} \quad (20)$$

If this condition is not satisfied the electrode will not remove all the B that reaches it and some B will be lost by reaction with Y in the bulk of the solution. This is a severe condition as most electrochemical rate constants are less than  $10^{-2} \text{ cm s}^{-1}$ . The condition in equation (14) with the value for  $X_G$  in equation (17) means that the kinetics of the reaction of B with Y must satisfy the condition,

$$k[Y] \sim 40 \text{ s}^{-1} \quad (21)$$

But the concentration of Y must be at least twice the concentration of B generated in the light. This is to ensure that there is no concentration polarisation at the dark electrode. Hence for the optimum case,

$$[Y] > 2[B] \sim 10^{-2}[A] \sim 10^{-3} \text{ M} \quad (22)$$

Substitution in equation (21) gives  $k < 4 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ . Thus from equations (19) to (21) we may summarise the contradictory requirements for the optimum case: (1) the species, A, must be rather soluble,  $[A] > 0.1 \text{ M}$ ; (2) the electron transfer reaction of the A,B couple on the illuminated electrode must be very rapid,  $k' > 10^{-1} \text{ cm s}^{-1}$ ; (3) considering the thermodynamic driving force corresponding to  $\Delta E$  of 1.1 V, the electron transfer reaction of B + Y must be sluggish:  $k < 4 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ ; (4) the electron transfer reaction that forms B and Y from  $A^* + Z$  must be rapid. Electron transfer reactions that obey the Marcus theory<sup>7</sup> are unlikely to satisfy all the conditions 2, 3 and 4 (S. W. Feldberg, personal communication).

The detailed treatment presented elsewhere<sup>2</sup> allows the optimum conditions and the power that can be produced for any particular system to be calculated. For instance, if we relax the condition of equation (14), so that  $X_e = X_G = X_k$  then we find that  $[A] \sim 10^{-2} \text{ M}$ ;  $k' > 10^{-2} \text{ cm s}^{-1}$ ;  $k < 4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ .

It may be possible to find a system that satisfies these conditions. For this case the power is only reduced by a factor of 2 from the optimum to about 9% and

$$W_{\max}/A = 70 \text{ W m}^{-2} \quad (23)$$

It is clear that the processes occurring in a photogalvanic cell are controlled by the photochemistry, homogeneous kinetics, mass transfer and electrochemical kinetics of the system. The power developed depends on the concentrations of all the species, the intensity of illumination, diffusion lengths at the electrode, the electrode kinetics, the spacing of the electrodes and the load on the cell. The optimum performance of the cell can only be obtained by carefully selecting the best values for all these variables.

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1. Albery, W. J. & Archer, M. D. *J. electroanal. Chem.* (in the press).
2. Albery, W. J. & Archer, M. D. *J. electroanal. Chem.* (in the press).
3. Albery, W. J., Archer, M. D. & Egdell, R. G. *J. electroanal. Chem.* **82**, 199 (1977).
4. Data from proposed AM2 spectral distribution in Interim Solar Cell Testing Procedures for Terrestrial Applications 13 (NASA, 1975).
5. Gates, D. M. *Science* **151**, 523 (1966).
6. Archer, M. D. *Solar Energy* (in the press).
7. Marcus, R. A. *J. chem. Phys.* **43**, 679 (1965).

# Cordilleran Benioff zones

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*Ages of late Mesozoic–early Cainozoic igneous rocks in south-western North America indicate a magmatic arc initiated near the continental margin, swept over 1,000 km northeastward, then swept back, all in 110 Myr. The sweep in and return are interpreted as due to flattening of a Benioff zone to  $<15^\circ$  followed by rapid collapse.*

THE geometric relationship between trench, dipping Benioff zone, and magmatic arc is well established in plate tectonics<sup>1–4</sup>. In active arc–trench systems, distance from the trench axis to the main volcanic front or magmatic arc axis varies from as little as 75 km to over 400 km<sup>5,6</sup>. Much of this variation is due to the width of the accretionary prism which seems to control the initial angle of descent of subducting slabs<sup>4,5</sup>. Once the slab is below a depth of 25–75 km the angle of descent usually steepens, but the descent angle of deeper segments of Benioff zones can range from  $90^\circ$  to as little as  $15^\circ$  (ref. 7). Surface manifestation of arc activity can extend over some width and stands between 100 km and 300 km above the Benioff zone<sup>6</sup>. Based on this geometry, the distance the magmatic arc activity can lie inboard from the trench is controlled by the dip-angle of the descending slab.

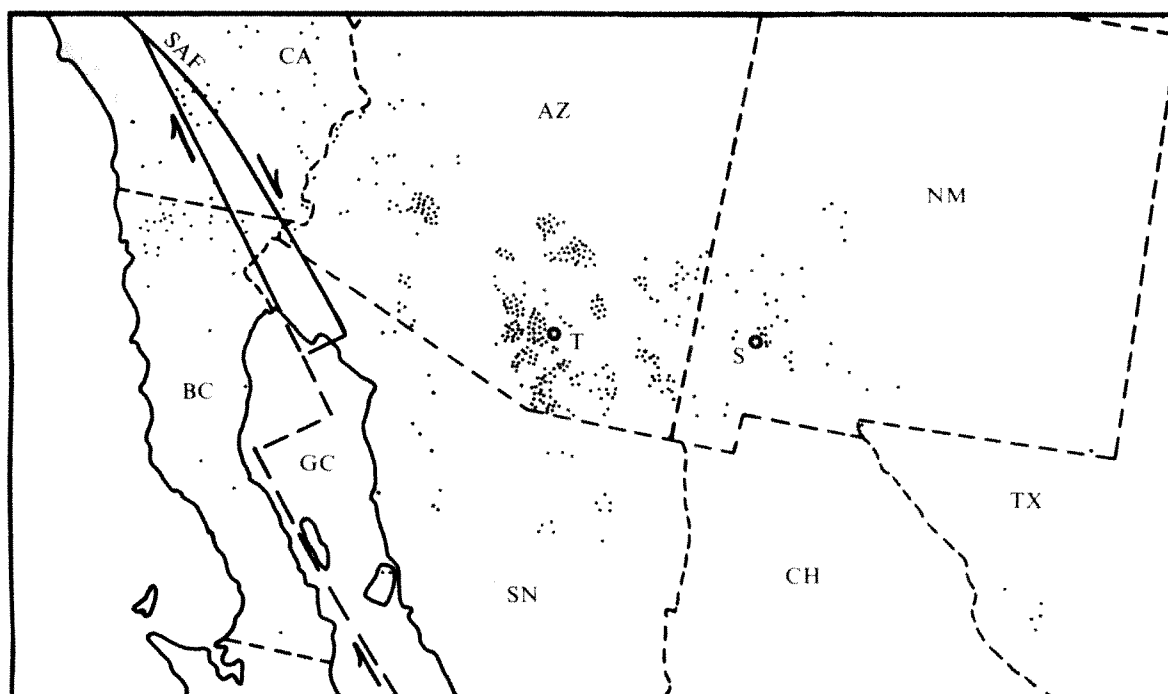
Extrapolation of this model back through Mesozoic time in the North American Cordillera<sup>8–10</sup> has not been universally accepted. Here, igneous activity of arc type reached inboard in excess of 1,000 km from assumed marginal trenches and many have doubted the existence of resulting low-dipping Benioff

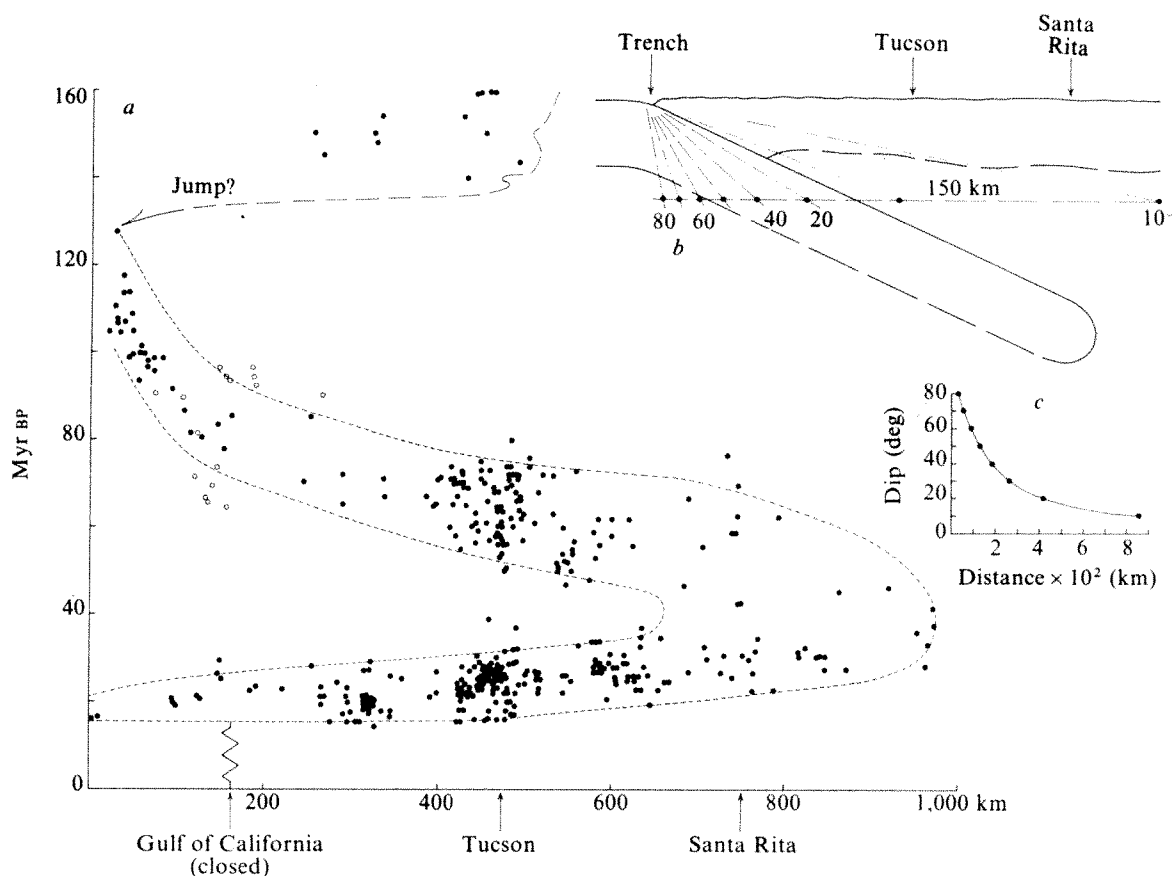
zone geometry<sup>11–13</sup>. We propose that widespread magmatic activity of arc type in southwestern North America during late Mesozoic to middle Tertiary time is consistent with Benioff zone geometry, and we show evidence that inferred changes in angle of Benioff zone dip can be tracked in time.

## Magmatic arc activity

Figure 1 shows the distribution of all available radiometric ages on igneous rocks from southern California, Arizona, New Mexico and west Texas in southwestern United States with a few additional ages from northern Baja California and Sonora in Mexico. The ages range from 160 Myr to about 15 Myr. The dated rocks have chemical compositions normally considered of magmatic arc type and include both volcanic and plutonic occurrences. The age dates are mostly by the K/Ar method supplemented with a few by Rb/Sr and U/Pb methods. Each dated occurrence has been examined for geological setting and method used. All available ages were used on Fig. 1 with the following exception: in the case of plutonic rocks from the Peninsular batholith in the western part of the region, multiple intrusion has caused extensive resetting of K/Ar ages<sup>14</sup>. As a result, only concordant biotite–hornblende pairs were used. In the absence of U/Pb ages concordant hornblende–biotite K/Ar pairs have been shown to be more reliable in belts of concentrated prolonged batholith emplacement<sup>15</sup>. Further east where plutonism was more dispersed single mineral dates were used. Throughout the region dates on volcanic rocks were considered accurate, providing they were geologically consistent and the method used indicated reliability.

**Fig. 1** Distribution of radiometric age determinations in southwestern United States and adjacent northwestern Mexico. Published dates from refs 14, 18–20, 44, 45. S. B. Keith and S. J. Reynolds compiled dates, lists and maps which are available from P. Damon, University of Arizona. Unpublished ages taken from refs 23, 44, and P. Damon, R. Nielson and W. Rehrig personal communication. T, Tucson; S, Santa Rita; SAF, San Andreas Fault; CA, California; AZ, Arizona; NM, New Mexico; BC, Baja California; GC, Gulf of California; SN, Sonora; CH, Chihuahua; TX, Texas.





**Fig. 2** *a*, Distribution of radiometric ages from Fig. 1 plotted as a function of time. Dates projected into a line passing through Tucson, Arizona, and Santa Rita, New Mexico after closure of Gulf of California.  $\circ$ , single mineral K/Ar ages on plutons in southeastern California. *b*, Hypothetical arc-trench system scaled to southwestern North America. Various dip-angles of Benioff zones shown with points of intersection with 150 km depth line. *c*, Graphical representation of function controlling distance from trench to magmatic arc with varying Benioff zone dip angles.

Figure 2a shows the distribution of radiometric ages plotted as a function of time. To construct this figure, the Gulf of California was first closed, bringing dates south-west of the San Andreas Fault about 300 km southeastward relative to dates north-east of the fault. This reconstruction restores the age distribution to a pattern more like the original distribution before post-14 Myr opening of the Gulf of California and displacement on the San Andreas Fault. A line was then drawn from the western edge of the continent through Tucson, Arizona and Santa Rita, New Mexico, approximately perpendicular to the restored continental margin and the assumed late Mesozoic-mid-Tertiary trench. All the ages on Fig. 1 were then projected on to this line to produce Fig. 2a.

The scattering of ages at the top of Fig. 2a is the youngest part of a group of ages which represent a north-west-south-east trending magmatic arc across southern Arizona during Jurassic time<sup>16</sup>. This arc was extinguished in latest Jurassic time. Arc activity then seems to have jumped south-west towards the supposed site of the trench in early Cretaceous time. This jump established an arc near the continental margin and produced the Peninsular batholith of southern California-northern Baja California<sup>14,17</sup>.

Ages on the Peninsular batholith form a cluster at the far western edge of Fig. 2a ranging from 125 Myr to about 85 Myr. The dates shown are mostly K/Ar concordant biotite-hornblende pairs<sup>18-20</sup>, and they define an array which is oldest in the west and younger to the east across the batholith. Silver *et al.*<sup>17</sup> report a similar, but slightly older, trend in U/Pb ages. It can be seen from Fig. 2a that the magmatic pulse, apparently of just over 20 Myr duration, continues its sweep eastward, progressively decreasing in age. It eventually crosses the entire southwestern Cordillera, reaching central New Mexico 1,000

km inboard from the assumed trench about 40 to 55 Myr ago. Anderson and Silver<sup>21</sup> report a similar, but presumably slightly older, trend in U/Pb ages from Baja California across Sonora.

At its most easterly limit near 40 to 55 Myr, the magmatic pulse is scattered and somewhat diffuse. It then starts a rapid return migration, sweeping back across the same terrain over which it had earlier advanced, reaching southern California 15 to 20 Myr ago. Thus, in a period of just over 100 Myr, arc magmatism swept continuously inboard 1,000 km, then swept back to where it had originated. The dashed lines on Fig. 2a are a gross envelope superimposed on the data bracketing the magmatic arc distribution. Since most of the plutonic ages plotted are by the K/Ar method, they are believed to represent times when the igneous rocks passed through cooling temperatures somewhat less than 'emplacement' temperatures. We note, however, that the few published U/Pb ages fall within the envelope, and we assume that the average emplacement age for the magmatic arc at any given time probably falls somewhere within the envelope as well. At about 15 Myr, the character of igneous activity of the entire region analysed abruptly changed. The chemistry became distinctly bi-modal, with much basalt, and was associated with Basin and Range rifting<sup>22,23</sup>, initiation of movement on the San Andreas Fault, and eventual opening of the Gulf of California.

### Analysis and interpretation

Figure 2b shows a hypothetical arc-trench system scaled to a geometry likely to have existed in southwestern North America during mid-Cretaceous to mid-Tertiary time, with various Benioff zone dip-angles ranging from 80° to 10°. Also shown are distances inboard from the trench where the Benioff zone intersects the 150 km depth line. This depth is presumed to lie

below the locus of magmatic arc activity at the surface<sup>6</sup>. The function controlling the position of the magmatic arc axis with varying Benioff zone dip is: distance = depth  $\times \cot \theta$ , where  $\theta$  is the dip. In other words, with decreasing dip the distance from the trench to the arc increases ever more rapidly Fig. 2c.

We suggest that the pattern of ages on Fig. 2a reflects the progressive flattening of a Benioff zone beneath southwestern North America from about 130–110 Myr to about 55–40 Myr, followed by a rapid steepening of dip from 40 Myr to about 15 Myr. This decreasing then increasing Benioff zone dip first swept a magmatic arc 1,000 km inboard from the trench until 55–40 Myr, then swept it back towards the continental margin by about 20 Myr.

Figure 3 is derived from Fig. 2a and shows the change in Benioff zone dip as a function of time beneath southwestern North America based on a best-fit curve through the centre of the envelope enclosing the age dates. Figure 4, also derived from Fig. 2a, is the value of the first derivative of the age distribution function and shows the velocity of the magmatic arc locus with respect to the trench as it swept inboard then back between 130 and 15 Myr. This velocity apparently attained almost 3 cm yr<sup>-1</sup> on the sweep in, collapsed to zero at the bend near 45 Myr, then increased to over 4 cm yr<sup>-1</sup> on the return. The duration of arc activity at any given place averages over 20 Myr during the sweep eastward and is somewhat less on the sweep back. The width of arc activity at any given time is initially narrow at steep angles of dip (about 100 km), but very wide (over 600 km) at low angles of dip or during periods of rapid migration.

## Discussion

If our interpretation that shifting magmatic arc patterns in southwestern North America are due to changes in dip of Cordilleran Benioff zones is correct, some explanation must be sought for the changing dips. Luyendyk<sup>24</sup> suggested that rates of convergence at subduction zones influenced Benioff zone dip with his formula: dip = arc sin  $V_z/V_0$ , where  $V_z$  is the natural rate a slab sinks due to gravity, and  $V_0$  is the rate of convergence. Thus, the higher the rate of convergence the lower the angle of dip. It has also been suggested that an actively driving continental plate, with a subduction zone dipping beneath the leading edge, pushes the subduction zone ahead, over-riding and entraining the Benioff zone below and decreasing the dip<sup>25</sup>. Other factors, such as age (temperature) and emboliment of aseismic ridges, might reduce slab density and influence Benioff zone dip<sup>26</sup>. Rates of convergence between an actively driving North America plate and Farallon plate may have increased during late Cretaceous–early Tertiary time, flattening the Benioff zone beneath western North America. Some time between 40–55 Myr the rate slowed, causing increase in Benioff zone dip and the resulting return sweep between 40 and 20 Myr.

Plate reconstructions based on assumed movement of Pacific and African plates over hotspots in the mantle since 135 Myr, although still suspect, yield vector subtraction derived convergent rates between North America and Farallon plates<sup>27,28</sup>. From 80 Myr to about 45 Myr the computed rates of convergence reach values in excess of 12 cm yr<sup>-1</sup>. The age of the bend in the Hawaii–Emperor seamount chain is placed at about 45 Myr (refs 29, 30) and is assumed to mark a change in direction of Pacific plate motion from northward to more north westward. After the change in direction, convergent rates between the two plates fall to less than 8 cm yr<sup>-1</sup>. We note that the age of the Hawaii–Emperor bend is the same as the age of the bend in Fig. 2a, and that high derived convergent rates correlate with the sweep inboard during Laramide time while the lower rates of post-45 Myr time correlate with the sweep back during mid-Tertiary time. Using Luyendyk's formula and a rate of convergence of 12 to 14 cm yr<sup>-1</sup>, the Benioff zone dip attains 20°. Slower rates of convergence between 6 and 8 cm yr<sup>-1</sup> yield dips from 40° to 60°. The high

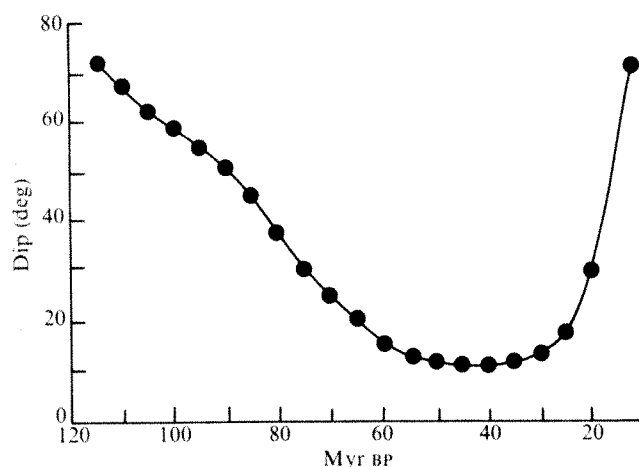


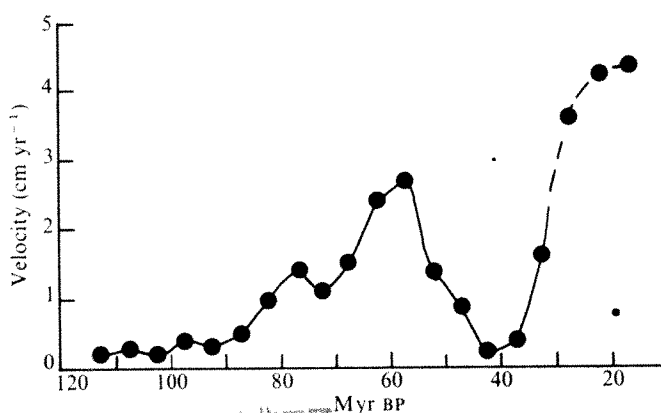
Fig. 3 Benioff zone dip angle as a function of time beneath southwestern North America. Graph derived from best-fit curve through data of Fig. 2a. A 15% post-15 Myr extension due to Basin and Range faulting has been removed from Fig. 2 to construct Fig. 3.

rates of convergence, coupled with a low-dipping Benioff zone during Laramide orogeny are thought to explain extensive deep-seated compressive thrusting which extended as far inboard from the trench as did the arc magmatism<sup>27,31</sup>.

The sweep in from 130 Myr to about 45 Myr includes two classic orogenic episodes in Cordilleran tectonics: the Sevier orogeny during mid-Cretaceous to about 80 Myr and the Laramide orogeny from 80 Myr to about 45 Myr (refs 9, 32, 33). The return sweep between 40 Myr and about 15 Myr includes what has been termed the mid-Tertiary orogeny<sup>34,35</sup>, or the ignimbrite flare-up<sup>28</sup>. In terms of magmatic arc activity, these 'orogenies' are here seen as a continuum broken only by the turn-around near 45 Myr which ended Laramide events and initiated the mid-Tertiary ignimbrite flare-up in southern Arizona. We note the latter part of the sweep inboard (Laramide time) produced a major percentage of North American copper porphyry deposits in southern Arizona, New Mexico, and Sonora. The return sweep across the same region produced essentially none.

We have preliminary evidence that the shifting magmatic arc patterns described for the southwestern Cordillera are related to similar patterns further north. Arc activity in the Sierra Nevada and Idaho batholiths began to disappear soon after 80 Myr (refs 19, 35). Just afterward a very sparse magmatic pulse swept eastward and rapidly died, producing the well

Fig. 4 Velocity of magmatic arc axis as a function of time. Graph derived from best-fit curve through data of Fig. 2a. A 15% post-15 Myr extension due to Basin and Range faulting has been removed from Fig. 2 to construct Fig. 4.





known Laramide 'igneous gap' between about 70 Myr and 55 Myr throughout the north-west and west-central United States<sup>36,10</sup>. At about 55 Myr the north-west erupted violently with wide-spread Challis-Absaroka volcanism and shallow plutonism<sup>36</sup>. This pattern then swept out of Washington, Idaho, Montana, and Wyoming, and moved progressively southwestward across western Utah and Nevada as the ignimbrite flare-up of the Great Basin between 40 and 20 Myr (refs 37-39). We interpret the sweep in and rapid extinguishing of arc activity as the result of the same flattening of a Benioff zone we describe to the south, the only difference being that in the north the dip became so shallow arc activity was impossible. The igneous gap extending from southern Ecuador to southern Peru, accompanied by an essentially horizontal Benioff zone<sup>40</sup>, is the present-day analogue. The Challis-Absaroka flare-up and subsequent return sweep of arc activity southwestward across the Great Basin would be the same return sweep we describe to the south and due to the same cause—a rapid increase in dip. Seen this way, the mid-Tertiary outburst of ignimbrites in the central and southern Cordillera becomes more comprehensible. Lipman *et al.*<sup>10</sup> called the outburst a magmatic arc related to subduction. We agree with this interpretation, and add that with the better resolution of time-space plots based on extensive radiometric dating the volcanism can be further seen as the rapid retrograde sweep of a magmatic arc toward the trench resulting from gravitational collapse and steepening of a Tertiary Benioff zone. Calling on complex back-arc diapirs<sup>41</sup> to explain mid-Tertiary volcanism in the Great Basin now seems unnecessary.

The data presented here suggest arc activity in southwestern North America was essentially continuous between 130 Myr and about 15 Myr. If a Kula-Farallon triple junction migrated up the North America plate margin during late Cretaceous-early Tertiary time, as suggested from some reconstructions<sup>42</sup>, we do not see evidence of its passage. The data seem more consistent with, but do not prove, continuous subduction of the Farallon plate during this time. Progressive destruction of the Pacific-Farallon spreading centre and Mendocino fracture zone along the North America plate margin during late Tertiary time<sup>42</sup> has bearing on interpretation of the youngest ages in the data array. Most reconstructions place this event between 30 Myr and 20 Myr, with initial contact somewhere along the coast of northernmost Baja California<sup>43</sup>. It is not certain to what degree the last part (25 Myr to 15 Myr) of the post-40 Myr collapse and rapid steepening of the Benioff zone was due to cessation of subduction caused by the change in plate geometry. Regardless, an arc activity delay-time of 5-10 Myr after slab truncation is predictable<sup>44</sup>.

The rapid retrograde migration of arc activity toward the trench after 40 Myr does not require excessive sinking rates

of the subducting slab. For example, between 40 Myr and 20 Myr the increase in dip-angle was about 20° (Fig. 3). This implies an approximate average vertical descent rate of about 1 cm yr<sup>-1</sup>. From 20 Myr to 15 Myr the increase in dip-angle was about 40° which yields an average sinking rate of about 5 cm yr<sup>-1</sup>. This 5 cm yr<sup>-1</sup> is the velocity Luyendyk<sup>84</sup> gives the natural vertical sinking rate ( $V_z$ ) of a slab due to gravity. This suggests the slab may actually have been detached and freely sinking after  $\pm$  25 Myr due to destruction of the East Pacific spreading centre and initiation of the San Andreas transform<sup>42</sup>.

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- McKenzie, D. P. & Parker, R. L. *Nature* **216**, 1276-1280 (1967).
- Isacks, B., Oliver, J. & Sykes, L. R. *J. geophys. Res.* **73**, 5855-5899 (1968).
- Dickinson, W. R. *Rev. Geophys.* **8**, 813-860 (1970).
- Karig, D. E. & Sharman, G. F., III *Geol. Soc. Am. Bull.* **86**, 377-389 (1975).
- Dickinson, W. R. *J. geophys. Res.* **78**, 3376-3389 (1973).
- Hatherton, T. & Dickinson, W. R. *J. geophys. Res.* **74**, 5301-5310 (1969).
- Isacks, B. & Molnar, P. *Rev. Geophys. Space Sci.* **9**, 103-174 (1971).
- Hamilton, W. *Geol. Soc. Am. Bull.* **80**, 2409-2430 (1969).
- Coney, P. J. *Nature* **233**, 462-465 (1971).
- Lipman, P. W., Prostka, J. H. & Christiansen, R. L. *Science* **174**, 821-825 (1971).
- Gilluly, J. *Geol. Soc. Am. Bull.* **82**, 2383-2396 (1971).
- Lowell, J. D. *Econ. Geol.* **69**, 601-617 (1974).
- Woodward, L. A. *Geology* **2**, 570 (1974).
- Gastil, R. G., Phillips, R. P. & Allison, E. C. *Geol. Soc. Am. Mem.* **140**, 170 (1975).
- Lanphere, M. N. & Reed, B. L. *Geol. Soc. Am. Bull.* **84**, 3773-3782 (1973).
- Hayes, P. T., Simons, F. S. & Raup, R. B. *U.S. Geol. Surv. Bull.* **1194-M**, 9 (1965).
- Silver, L. T., Early, T. O. & Anderson, T. H. *Geol. Soc. Am. Cordilleran Sec. Meet.* 375-376 (1975).
- Gastil, R. G. & Krummenacher, D. *Geol. Soc. Am. Bull.* **88**, 189-198 (1977).
- Armstrong, R. L. & Suppe, J. *Geol. Soc. Am. Bull.* **84**, 1375-1392 (1973).
- Krummenacher, D., Gastil, R. G., Bushee, J. & Doupoint, J. *Geol. Soc. Am. Bull.* **86**, 760-768 (1975).
- Anderson, T. H. & Silver, L. T. *Geol. Soc. Am., Rocky Mountain Meet.* 484 (1974).
- Damon, P. E. & Mauger, R. L. *Trans. S.M.E.* **235**, 99-112 (1966).
- Eberly, L. D. & Stanley, T. B. Jr. *Geol. Soc. Am. Bull.* (in the press).
- Luyendyk, B. P. *Geol. Soc. Am. Bull.* **81**, 3411-3416 (1970).
- Coney, P. J. in *Implications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) **2**, 713-727 (Academic, London, 1973).
- Kelleher, J. & McCann, W. J. *geophys. Res.* **81**, 4885-4896 (1976).
- Coney, P. J. *New Mexico geol. Soc. Spec. Pub.* **4**, 5-10 (1976).
- Coney, P. J. *Geol. Soc. Am. Spec. Pap.* (in the press).
- Morgan, W. J. *et al. Geol. Soc. Am. Mem.* **132**, 7-22 (1972).
- Clague, D. A. & Jarrard, R. D. *Geol. Soc. Am. Bull.* **84**, 1135-1154 (1973).
- Burchfiel, B. C. & Davis, G. A. *Nature* **260**, 693-694 (1976).
- Armstrong, R. L. *Geol. Soc. Am. Bull.* **79**, 429-458 (1968).
- Heldrick, T. L. & Titley, S. R. *24th Int. Geol. Cong.* **3**, 740 (1976).
- Damon, P. E. *et al. A. Prog. Rep. A.E.C. COO-* 689-42 (1964).
- Armstrong, R. L., Taubeneck, W. H. & Hales, P. O. *Geol. Soc. Am. Bull.* **88**, 397-411 (1977).
- Armstrong, R. L. *Northwest Geol.* **3**, 1-15 (1974).
- Stewart, J. H., Moore, W. J. & Zeitz, I. *Geol. Soc. Am. Bull.* **88**, 67-77 (1977).
- Armstrong, R. L. & Higgins, R. E. *Geol. Soc. Am. Bull.* **84**, 1095-1100 (1973).
- Noble, D. C. *Earth planet. Sci. Lett.* **17**, 142-150 (1972).
- Stauder, W. J. *J. geophys. Res.* **80**, 1053-1064 (1975).
- Scholz, C. H., Barazangi, M. & Sbar, M. L. *Geol. Soc. Am. Bull.* **82**, 2979-2990 (1971).
- Atwater, T. *Geol. Soc. Am. Bull.* **81**, 3513-3536 (1970).
- Atwater, T. & Molnar, P. *Stanford Univ. Publ. Geol. Sci.* **13**, 136-149 (1973).
- Snyder, W. S., Dickinson, W. R. & Silberman, M. L. *Earth planet. Sci. Lett.* **32**, 91-106 (1976).
- Elston, W. E. & Northrop, S. A. *New Mexico Geol. Soc. Spec. Pub.* **5**, 151 (1976).

# Regulation of protein synthesis, intracellular electrolytes and cataract formation *in vitro*

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*Control of protein synthesis is associated with changes in the ratio of intracellular Na<sup>+</sup> to K<sup>+</sup> in the cultured embryonic chick lens. Correlations of intracellular Na<sup>+</sup>/K<sup>+</sup> ratios with crystallin synthesis and cataract formation in vitro suggest that the Na<sup>+</sup>/K<sup>+</sup> ratio may have an important role in the regulation of protein synthesis during cataractogenesis.*

CATARACTS, or lens opacities, represent a primary cause of seriously impaired vision and even blindness. At present, a cataract that has progressed beyond the initial stages cannot be reversed, forcing the patient to have the affected lens removed in order to restore clear vision. Cataracts are initiated by or associated with a variety of factors, including physical trauma, ageing, metabolic stress, hereditary defects, disease, ionising radiation and microwaves<sup>1</sup>. Although the

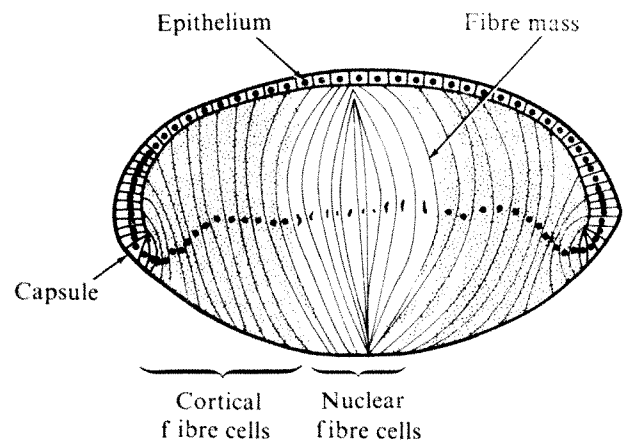
mechanism of cataractogenesis has not been definitively established for any one type of lens opacity, considerable evidence indicates that osmotic imbalance<sup>2</sup> and protein aggregation<sup>3-5</sup> may play prominent parts.

We have reported<sup>6</sup> that cortical cataracts are formed within 3 h after explantation of the embryonic chick lens into a defined culture medium (Ham's F-10<sup>7</sup>) (see Fig. 1). This experimental cataract seems to be caused by the trauma of detaching the vitreous body, a gelatinous connective tissue matrix. The initiation of this experimental cataract is associated with a specific alteration in the synthesis of  $\delta$ -crystallin, the principal protein of the embryonic chick lens<sup>8</sup>.  $\delta$ -Crystallin represents 60–80 % of the protein present in these lenses<sup>9-13</sup> and can be resolved into two bands with molecular weight near 50,000 and 48,000 by electrophoresis in a sodium dodecylsulphate-urea-polyacrylamide gel<sup>14</sup>. Clear lenses synthesise the proteins in these two bands in a ratio of approximately 1 : 3 in favour of the lower molecular weight band. In vitreous-free lenses developing cortical opacities the ratio of synthesis of proteins in these two bands is reversed to approximately 3 : 1 in favour of the higher molecular weight band. This alteration in protein synthesis is associated only with the initiation of the cataract, since these  $\delta$ -crystallin polypeptides are synthesised in the normal ratio after the lens has remained in culture for 24 h, despite persistence of the cortical opacity<sup>6</sup>.

Here we explore the possibility that the alteration of  $\delta$ -crystallin synthesis in the cultured lens is due to an increase in the intracellular concentration of  $\text{Na}^+$  and a decrease in the intracellular concentration of  $\text{K}^+$ , since several reports have shown that cataracts are associated with  $\text{Na}^+$  influx and  $\text{K}^+$  efflux<sup>2,15-20</sup>. In addition, one investigation has shown that stripping the vitreous body from a cultured rabbit lens accelerates cataract formation<sup>21</sup> and causes an increase in  $\text{Na}^+$  and a decrease in  $\text{K}^+$  concentration within the lens<sup>22</sup>. Moreover, experiments with bacterial<sup>23</sup> and mammalian<sup>24</sup> cells have indicated the importance of  $\text{K}^+$  for the intracellular control of protein synthesis, and investigations of cell-free translation<sup>25</sup> have provided evidence that  $\text{Na}^+$  and  $\text{K}^+$  ions can discriminate between different mRNAs to allow preferential translation at different ionic strengths. Our data indicate the changes in the intracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$  cause the alteration of  $\delta$ -crystallin synthesis in the embryonic chick lens cultured without its vitreous body. This raises the possibility that the intracellular levels of these ions also contribute to the control of differential protein synthesis in other eukaryotic cells; this in turn may influence cellular growth, differentiation and pathogenesis.

### $\text{Na}^+$ and $\text{K}^+$ in cultured lenses with and without vitreous body

$\text{Na}^+$  and  $\text{K}^+$  concentrations were determined in the embryonic chick lens immediately after explantation, after 3 h of culture with its vitreous body attached, and after 3 or 24 h of culture with its vitreous body detached (Table 1). At the time of explantation, the concentrations of these ions were similar to those found for lenses of other species<sup>26</sup>, with  $\text{Na}^+$  being considerably lower than  $\text{K}^+$ . The  $\text{Na}^+$  and  $\text{K}^+$  concentrations remained the same after the lenses were cultured for 3 h with their vitreous bodies; these lenses with attached vitreous body have been shown to be transparent and synthesise the proteins comprising the two bands of  $\delta$ -crystallin in the normal ratio of 1 : 3 in favour of the lower molecular weight band<sup>6</sup>. By contrast, after removal of the vitreous body, the  $\text{Na}^+$  concentration increased approximately sixfold and the  $\text{K}^+$  concentration decreased approximately 2.5-fold within 3 h; these vitreous-free lenses have been shown to possess cortical cataracts and synthesise the proteins of the two  $\delta$ -crystallin bands in a ratio of approximately 3 : 1 in favour of the higher molecular weight band<sup>6</sup>.



**Fig. 1** Diagrammatic representation of a cross section of a 15-d-old embryonic chick lens. The nuclei in the centre of the fibre mass (nuclear fibre cells) are starting to become pycnotic and will eventually disintegrate. The stippled zone represents the area which becomes opaque when the lens is cultured after its vitreous body has been removed from the posterior surface. The vitreous body (not shown) occupies a major part of the eye and consists of an avascular, viscous connective tissue matrix containing fibroblasts, collagenous material and other proteins, mucopolysaccharides and organic substances.

After 24 h *in vitro* without the vitreous body, the  $\text{Na}^+$  concentration had returned to nearly its initial value at explantation; the vitreous-free lens after 24 h *in vitro* has been shown to still have the cortical cataract, but to return to a relatively normal pattern of  $\delta$ -crystallin synthesis<sup>6</sup>. Since the intracellular  $\text{K}^+/\text{Na}^+$  ratio and pattern of  $\delta$ -crystallin synthesis are markedly different in the lenses cultured with or without the vitreous body, we were encouraged to pursue the relationship between the intracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$  and the ratio of synthesis of the  $\delta$ -crystallin polypeptides.

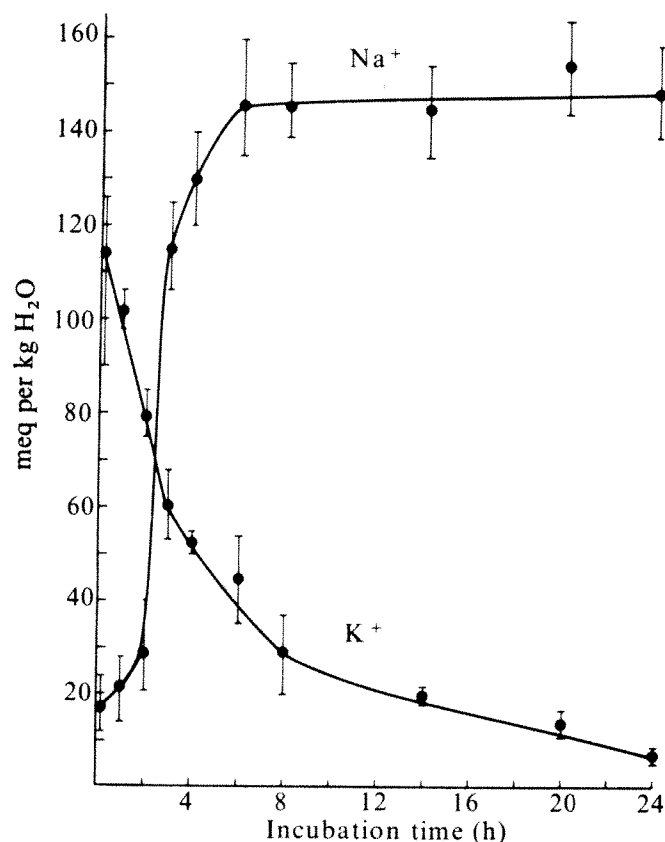
### Effect of ouabain on protein synthesis in cultured lenses

In order to alter the intracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$  without removing the vitreous body, explanted lens-vitreous preparations were treated with ouabain, an inhibitor of  $\text{Na}^+/\text{K}^+$  ATPase activity<sup>27</sup>. This enzyme is responsible for the permeability of  $\text{Na}^+$  and  $\text{K}^+$  in eukaryotic cells. Inhibition of its activity with ouabain is known

**Table 1**  $\text{K}^+$  and  $\text{Na}^+$  concentrations in embryonic chick lenses

	mEq per kg $\text{H}_2\text{O}$		
	$\text{K}^+$	$\text{Na}^+$	$\text{K}^+/\text{Na}^+$
Immediately after explantation	112 $\pm$ 11	17 $\pm$ 3	6.6
Cultured 3 h, + vitreous body	110 $\pm$ 7	16 $\pm$ 6	6.9
Cultured 3 h, - vitreous body	40 $\pm$ 3	84 $\pm$ 9	0.5
Cultured 24 h, - vitreous body	47 $\pm$ 8	29 $\pm$ 18	1.6

Four to eight lenses from 15-d-old chick embryos with or without their vitreous body were cultured in plastic tissue culture dishes (Falcon, 60  $\times$  15 mm) containing 10 ml of Ham's F-10 medium supplemented with fructose (848 mg per 100 ml); all assays were performed on lenses stripped of their vitreous body. Each determination represents an average of five to eight experiments. The lenses were washed quickly in groups of four with deionised water, placed in pyrex test tubes (previously soaked overnight in concentrated nitric acid to remove adhering  $\text{Na}^+$ ), dried at 75  $^\circ\text{C}$  for 24 h, dissolved in 100  $\mu\text{l}$  of concentrated nitric acid for several hours at room temperature and diluted with 40 ml of deionised water.  $\text{K}^+$  was determined by flame photometry and  $\text{Na}^+$  by atomic absorption spectrometry (Perkin-Elmer model 603). The average water content of the embryonic lenses was 85 % of their total weight. Corrections were made for ions in the extracellular space of the lens, which was determined by experiments with  $^3\text{H}$ -inulin.



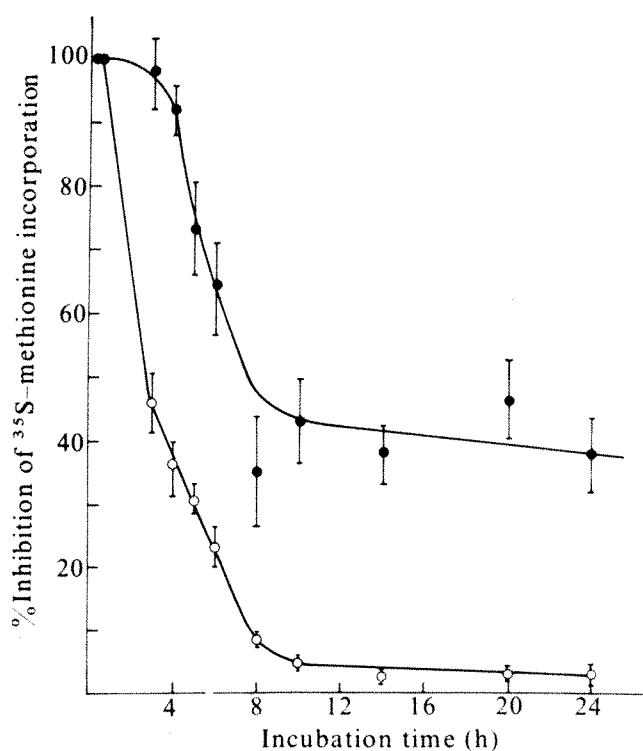
**Fig. 2** Effect of ouabain on the concentrations of Na<sup>+</sup> and K<sup>+</sup> in cultured embryonic chick lenses. Each point on the graph represents an average of three experiments; the vertical lines indicate the range of values obtained in the three tests. The lenses were cultured with their vitreous body and analysed for Na<sup>+</sup> and K<sup>+</sup> as given in Table 1.

to increase the Na<sup>+</sup> and decrease the K<sup>+</sup> concentration in cultured lenses of other species<sup>28-31</sup>. Ouabain caused a reciprocal change in the relative concentrations of the ions of the order of tenfold for each ion in the cultured lenses (Fig. 2). The ouabain-treated embryonic lens-vitreous preparations developed a diffuse opacity covering the entire epithelium and the most anterior aspect of the fibre mass. The bulk of the fibres remained clear, however, even after 24 h *in vitro*, and did not develop cortical cataracts unless the vitreous body was detached.

Incorporation of <sup>35</sup>S-methionine into protein was severely affected by ouabain treatment. Lenses labelled for 3 h with <sup>35</sup>S-methionine incorporated about 90% less isotope into their total protein after 24 h of preincubation with  $1 \times 10^{-4}$  M ouabain than after a comparable preincubation in the absence of the drug. The pattern of incorporation into  $\delta$ -crystallin was strikingly altered in lenses treated with ouabain. Incorporation of <sup>35</sup>S-methionine into the protein of the lower molecular weight  $\delta$ -crystallin band was inhibited by at least 95% after 8 h of culture with ouabain, while incorporation of the <sup>35</sup>S-methionine into the protein of the higher molecular band was only inhibited 50–60% (Fig. 3). This greater inhibition of methionine incorporation into the protein of the lower molecular weight band of  $\delta$ -crystallin persisted as long as ouabain was present in the culture medium. The staining patterns of the polyacrylamide gels, reflecting the absolute amount of protein present in each band, were similar in the preparations from lenses cultured with or without ouabain. The difference in <sup>35</sup>S-methionine incorporation into the proteins of the two  $\delta$ -crystallin bands was apparently not due to differential degradation of the newly synthesised polypeptides, since the protein in neither band was degraded in lenses labelled with <sup>35</sup>S-methionine

for 3 h in the absence of ouabain and subsequently incubated for 24 h in the presence of ouabain and the absence of protein synthesis (inhibited by  $10 \mu\text{g ml}^{-1}$  cycloheximide). Since the rate of the total uptake of <sup>35</sup>S-methionine into the lens did not decrease over the 24 h of culture (data not shown), the differential decrease of <sup>35</sup>S-methionine incorporation into protein seems to be due to differential inhibition of synthesis of the  $\delta$ -crystallin polypeptides.

It is important to note that the change in the ratio of synthesis of the  $\delta$ -crystallin polypeptides occurred in the epithelium and the fibre mass of the ouabain-treated lens, but was confined to the fibre mass in the vitreous-free lens. We believe that this difference in the localisation of the alteration of  $\delta$ -crystallin synthesis is due to a corresponding difference in the localisation of the changes in the levels of Na<sup>+</sup> and K<sup>+</sup>. The Na<sup>+</sup>,K<sup>+</sup> ATPase activity of the lens is located principally<sup>29,32,33</sup>, although apparently not exclusively<sup>34,35</sup>, in the epithelium. The ionic balance of the lens is thus thought to be governed by a pump-leak system<sup>36</sup>. Na<sup>+</sup> diffuses into the lens through the fibre mass at the posterior surface and is transported out of the lens through the epithelium at the anterior surface; by contrast, K<sup>+</sup> is transported into the lens through the epithelium and diffuses out of the lens through the fibre mass. Thus, detaching the vitreous body from the posterior surface may increase Na<sup>+</sup>



**Fig. 3** Effect of  $1 \times 10^{-4}$  M ouabain on the relative incorporation of <sup>35</sup>S-methionine into the proteins of the higher and lower molecular weight bands of  $\delta$ -crystallin. The lenses were cultured with their vitreous body as for Table 1. Each point on the graph represents an average of three experiments; the vertical lines indicate the range of values obtained in the three tests. Lenses were labelled individually for 3 h in 1 ml of medium containing 250  $\mu\text{Ci}$  of <sup>35</sup>S-methionine (New England Nuclear, 450 Ci mmol<sup>-1</sup>). One to 2  $\mu\text{g}$  of total homogenate protein was examined by electrophoresis in a discontinuous, 10% polyacrylamide gel containing 0.1% sodium dodecylsulphate and 8 M urea as described elsewhere<sup>14</sup>. The stained  $\delta$ -crystallin bands were cut from the gel, dissolved in 100  $\mu\text{l}$  of hydrogen peroxide and assayed for radioactivity by scintillation counting. The % inhibition of incorporation by ouabain into the protein of each band was calculated by dividing the amount of radioactivity in that band at each time point by the amount of radioactivity in the same band from lenses labelled for 3 h without ouabain immediately after explantation. ●, Higher molecular weight band; ○, lower molecular weight band.



diffusion into the lens without significantly changing the concentrations of  $\text{Na}^+$  and  $\text{K}^+$  in the epithelium. Ouabain treatment, however, should increase the  $\text{Na}^+$  concentration and decrease the  $\text{K}^+$  concentration in both the fibre mass and the epithelium by inhibiting the  $\text{Na}^+, \text{K}^+$  ATPase activity of the lens.

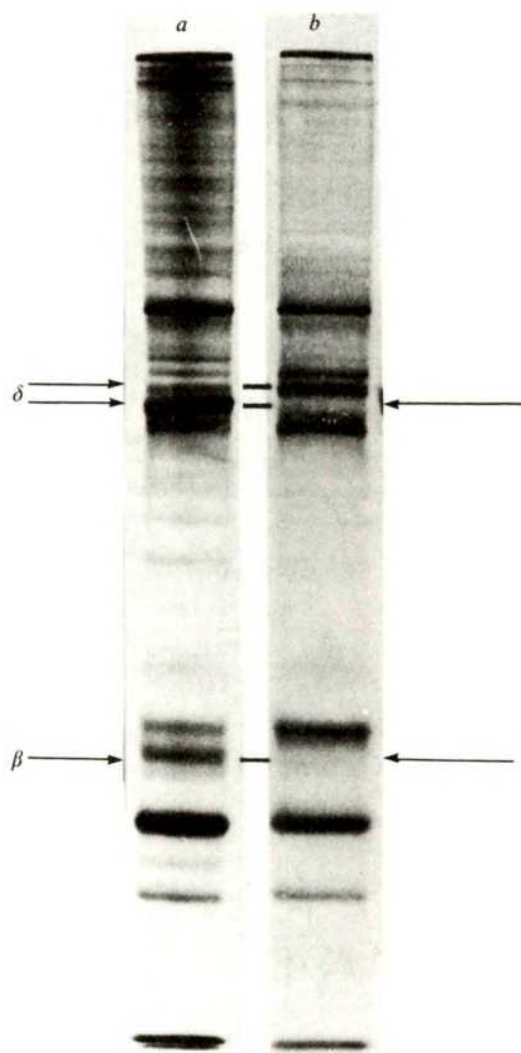
In addition to the alteration of  $\delta$ -crystallin synthesis, ouabain treatment also caused the differential inhibition of synthesis of other proteins in the cultured lenses. This was best observed when examining the isolated epithelium where  $\delta$ -crystallin represents a much smaller proportion of the total protein than in the fibre mass. The autoradiograms shown in Fig. 4 demonstrate the marked inhibition of synthesis of a  $\beta$ -crystallin component as well as that of the protein in the lower molecular weight  $\delta$ -crystallin band in lenses treated with ouabain. The synthesis of this  $\beta$ -crystallin polypeptide is also inhibited in lenses developing cortical cataracts in culture after their vitreous body has been removed. Inspection of the autoradiograms of Fig. 4 reveals a number of other differences in the relative amount of  $^{35}\text{S}$ -methionine incorporated into different bands of protein. Since equal amounts of radioactivity were applied to the gels, the synthesis of some bands of protein seems to be selectively stimulated by ouabain, but these actually represent proteins whose synthesis is not appreciably inhibited by the agent.

### Regulation of protein synthesis by $\text{Na}^+$ and $\text{K}^+$

To test the effect of  $\text{Na}^+$  and  $\text{K}^+$  on protein synthesis we examined cultured lenses with different intracellular concentrations of these ions. This was made possible by varying the amounts of  $\text{Na}^+$  and  $\text{K}^+$  in the culture medium of the lenses, which were permeable to these cations after treatment with ouabain. Determination of the intralenticular  $\text{Na}^+$  and  $\text{K}^+$  concentrations confirmed that the ouabain-treated lens was permeable to  $\text{Na}^+$  and  $\text{K}^+$ , and that these ions were at a similar concentration within the lens and in the medium. Tests with  $^3\text{H}$ -inulin, a compound which does not penetrate into cells, established that the ions were principally in the lens cells rather than in the extracellular space.

The incorporation of  $^{35}\text{S}$ -methionine into protein was sensitive to changes in the total intracellular concentration of  $\text{Na}^+$  and  $\text{K}^+$ .  $^{35}\text{S}$ -methionine incorporation was severely inhibited at combined  $\text{Na}^+$  and  $\text{K}^+$  concentrations above 200 mM or below 10 mM.  $\text{K}^+$  concentrations above 96 mM almost completely inhibited  $^{35}\text{S}$ -methionine incorporation into protein. These inhibitions of  $^{35}\text{S}$ -methionine incorporation into protein at different ionic strengths were apparently due to decreases in protein synthesis rather than  $^{35}\text{S}$ -methionine uptake since the total accumulation of radioactivity was not appreciably different in lenses cultured in the presence of ouabain in medium containing different concentrations of  $\text{Na}^+$  and  $\text{K}^+$ .

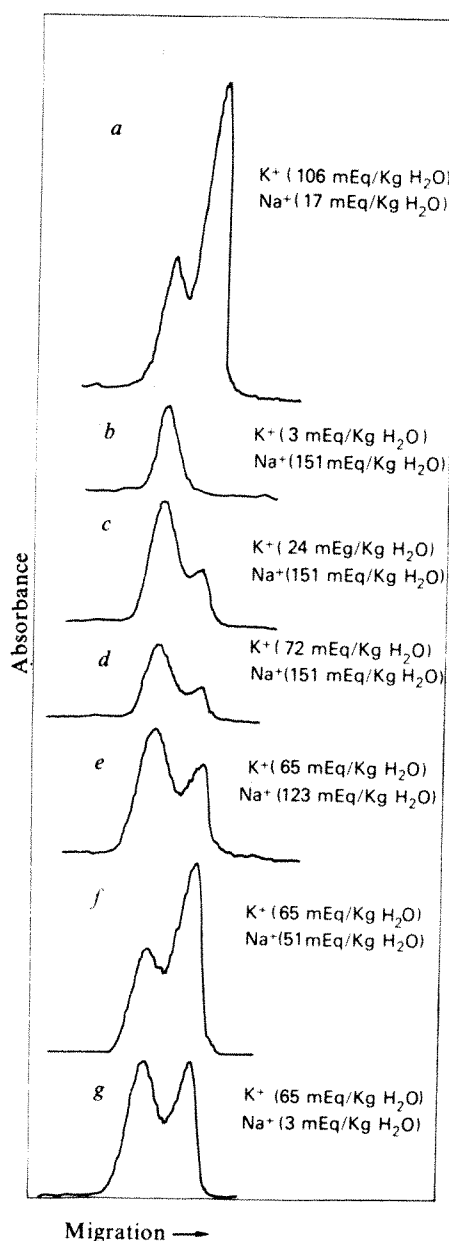
The most striking result of these tests was the sensitivity of the ratio of synthesis of the  $\delta$ -crystallin polypeptides to the variations in the concentrations of  $\text{Na}^+$  and  $\text{K}^+$ . The differential inhibition of synthesis of the protein in the lower molecular weight band of  $\delta$ -crystallin induced by ouabain (Fig. 5b) was partially overcome by increasing the concentration of  $\text{K}^+$  (Fig. 5c, d). Lowering the  $\text{Na}^+$  concentration also increased the relative amount of  $^{35}\text{S}$ -methionine incorporated into the protein in the lower molecular weight band of  $\delta$ -crystallin (Fig. 5e, f). The differential synthesis of the  $\delta$ -crystallin polypeptides does not seem to be simply a function of the total intracellular concentration of  $\text{Na}^+$  plus  $\text{K}^+$  because the protein in the lower molecular weight band was not synthesised at 154 mEq per kg of water of  $\text{Na}^+$  plus  $\text{K}^+$  (Fig. 5b) but was synthesised at higher (Fig. 5c-e) and lower (Fig. 5f, g) concentrations of intracellular  $\text{Na}^+$  plus  $\text{K}^+$ . Furthermore, the combined concentration of  $\text{Na}^+$  plus  $\text{K}^+$  in lenses cultured



**Fig. 4** Autoradiograms demonstrating the effect of ouabain on protein synthesis in the epithelium of the embryonic chick lens. Lenses were cultured in the absence (a) or presence (b) of  $1 \times 10^{-4}$  M ouabain for 24 h and then labelled with  $^{35}\text{S}$ -methionine for 3 h as given in Fig. 3. The epithelium was separated from the fibre mass and the proteins subjected to discontinuous polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate and urea<sup>14</sup>. The gels were stained and autoradiographed. The arrows on the right denote the almost complete inhibition of  $^{35}\text{S}$ -methionine incorporation into the proteins in the lower molecular weight  $\delta$ -crystallin band and into one  $\beta$ -crystallin band by ouabain. The staining patterns of the two samples were the same and were similar to the autoradiographic pattern of (a).

for 3 h with or without the vitreous body was the same, but the ratio of synthesis of the  $\delta$ -crystallin polypeptides has been shown to be different in the two cases<sup>6</sup> and varies with the ratio of the concentration of these ions (see Table 1). These results were not due to a differential processing of the higher molecular weight  $\delta$ -crystallin protein to a lower molecular weight form at different concentrations of  $\text{Na}^+$  and  $\text{K}^+$ , because there was no transfer of radioactivity from the higher to the lower molecular weight band in preparations from lenses labelled for 3 h in the presence of ouabain in the ionic conditions described in Fig. 5b and subsequently incubated for 2 d with ouabain in the absence of protein synthesis (inhibited by  $10 \mu\text{g ml}^{-1}$  cycloheximide) in the ionic conditions given in Fig. 5f. The differential inhibition of synthesis of the non- $\delta$ -crystallin proteins was also eliminated by adjusting the concentration of  $\text{Na}^+$  and  $\text{K}^+$  in the lenses treated with ouabain (data not shown). Thus, intracellular  $\text{K}^+/\text{Na}^+$  ratios greater than 1 generally allowed





**Fig. 5** Scans of autoradiograms demonstrating the effects of  $\text{Na}^+$  and  $\text{K}^+$  on the synthesis of the higher and lower molecular weight polypeptides of  $\beta$ -crystallin. Embryonic chick lenses were cultured for 24 h in the absence (a) or presence (b–g) of  $1 \times 10^{-4}$  M ouabain and labelled with  $^{35}\text{S}$ -methionine for 3 h as in Fig. 3, except that the  $\text{Na}^+$  and  $\text{K}^+$  concentrations in the medium were varied. Approximately equal amounts of  $\delta$ -crystallin (1–2  $\mu\text{g}$ ) were examined by electrophoresis and autoradiography, and the  $\delta$ -crystallin bands were scanned with a Quick Scan Jr densitometer (Helena Laboratories). The  $\text{Na}^+$  and  $\text{K}^+$  values were (in mEq per kg  $\text{H}_2\text{O}$ ): a,  $\text{K}^+$ , 106;  $\text{Na}^+$ , 17; b,  $\text{K}^+$ , 3;  $\text{Na}^+$ , 151; c,  $\text{K}^+$ , 24;  $\text{Na}^+$ , 151; d,  $\text{K}^+$ , 72;  $\text{Na}^+$ , 151; e,  $\text{K}^+$ , 65;  $\text{Na}^+$ , 123; f,  $\text{K}^+$ , 65;  $\text{Na}^+$ , 51; g,  $\text{K}^+$ , 65;  $\text{Na}^+$ , 3. These values represent intracellular levels of these ions which, except for (a), were similar to the concentrations in the medium, since ouabain made the lens permeable to  $\text{Na}^+$  and  $\text{K}^+$ . The concentrations of  $\text{Na}^+$  and  $\text{K}^+$  in Ham's F-10 medium (a) are 151 mEq per kg water and 3 mEq per kg water, respectively. Deficiencies in  $\text{Na}^+$  were compensated by adding choline chloride to the medium (e–g).

the normal ratio of synthesis of the proteins in the two  $\delta$ -crystallin bands, while  $\text{K}^+/\text{Na}^+$  ratios less than 1 favoured the synthesis of the protein in the higher molecular weight band of  $\delta$ -crystallin (see Table 1 also). The only deviation from this behaviour that we have observed is presented in Fig. 5g, where an extremely low  $\text{Na}^+$  concentration permitted more synthesis of the protein in the lower molecular

weight  $\delta$ -crystallin band than would have occurred at a slightly higher concentration of  $\text{Na}^+$ . The alteration of  $\delta$ -crystallin synthesis, then, is not entirely a simple function of the intracellular  $\text{Na}^+/\text{K}^+$  ratio.

## Discussion

These present data have implications with respect to the biology and pathology of the lens and the control of protein synthesis in general at the cellular and molecular levels. Experiments with cultured rabbit lenses showed that a disturbance of the normal lens-vitreous relationship alters the normal intralenticular  $\text{Na}^+$  and  $\text{K}^+$  concentrations<sup>22</sup>. Our experiments extend this observation to the embryonic chick lens and in addition demonstrate that the altered intracellular  $\text{Na}^+$  and  $\text{K}^+$  levels are correlated with differential changes in protein synthesis. It is important to note, however, that we do not know if the differential inhibition of protein synthesis is controlled directly or indirectly by the intracellular levels of  $\text{Na}^+$  and  $\text{K}^+$ . The  $\text{Na}^+$  and  $\text{K}^+$  concentrations of the lens are known to change during cataractogenesis in other species<sup>2,15–20</sup>, so it is possible that there are associated disturbances in protein synthesis which, except for the cultured chick lens<sup>6</sup>, have not yet been observed. It is not known whether such putative changes in protein synthesis might have a causal role in the cataractogenic process in some cases. The cortical cataract formed in the embryonic chick lens cultured without its vitreous body is not due to the alteration in  $\delta$ -crystallin synthesis because the same disturbance in synthesis takes place in lenses treated with ouabain but the cortical fibres remain clear. The lens epithelia, however, become cloudy in the ouabain-treated lenses.

Deviations from the normal pattern of polypeptide synthesis, such as demonstrated here, may have consequences extending to higher levels of protein structure. Lens crystallins may be particularly susceptible to changes in their native structure since they are aggregates of polypeptides<sup>8,37,38</sup>. The alteration in synthesis of the ratio of the  $\delta$ -crystallin polypeptides does indeed result in new forms of the native protein, which is composed of four subunits<sup>39</sup>. These new species of  $\delta$ -crystallin, to be reported elsewhere, are still tetramers but consist of different combinations of  $\delta$ -crystallin subunits. Clearly alterations in the structure of native proteins may also affect their function.

Our earlier experiments involving cell-free translation of purified  $\delta$ -crystallin mRNA suggest that the control of  $\delta$ -crystallin synthesis by  $\text{Na}^+$  and  $\text{K}^+$  is mediated at the translational level<sup>14</sup>. Nonetheless, we have not excluded the possibility that one of the  $\delta$ -crystallin bands is post-translationally derived from the other. This would be consistent with the observation that the two bands have similar (although not necessarily identical) tryptic peptides<sup>14,40</sup>. A post-translational modification of either band into the other would have to be associated with translation, since the bands do not interconvert after they have been synthesised<sup>14</sup>. Differential translation has been reported for immunoglobulin mRNAs<sup>41</sup>, HeLa cell mRNAs<sup>42</sup> and viral mRNAs<sup>43–45</sup> in cells under suboptimal conditions for protein synthesis. Evaluation of the literature of many different systems<sup>28,46–49</sup> indicates that translational control of protein synthesis generally occurs at the level of chain initiation. Thus, if the present control of  $\delta$ -crystallin synthesis by  $\text{Na}^+$  and  $\text{K}^+$  functions at the translational level, it is likely that this involves regulation of initiation. This would represent a very interesting example of translational control of protein synthesis in view of the apparent homogeneity of  $\delta$ -crystallin mRNA<sup>50</sup>.

It has been pointed out previously that the intracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$  may affect the absolute amount of macromolecular synthesis, and thus perhaps growth, in eukaryotic cells<sup>24</sup>. In view of the present data,

one may further speculate that the control of specific protein synthesis during differentiation may be affected by differences in the intracellular levels of  $\text{Na}^+$  and  $\text{K}^+$ . For example, the relative proportions of crystallins synthesised change during differentiation of the lens and differ in different compartments of the lens<sup>8,10-13,51</sup>. Possibly gradients of ions within the lens play a part in this regulation of crystallin synthesis.

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1. *Cataract and Abnormalities of the Lens* (ed. Bellows, J. G.) (Grune and Stratton, New York, 1975).
2. Kinoshita, J. H. *Invest. Ophthalmol.* **13**, 713-724 (1974).
3. Benedek, G. B. *Appl. Opt.* **10**, 459-473 (1971).
4. Spector, A. *Isr. J. med. Sci.* **8**, 1577-1582 (1972).
5. Jedziniak, J. A., Kinoshita, J. H., Yates, E. M., Hocker, L. O. & Benedek, G. B. *Invest. Ophthalmol.* **11**, 905-915 (1972).
6. Piatigorsky, J. & Shinohara, T. *Science* **196**, 1345-1347 (1977).
7. Ham, R. G. *Expl Cell Res.* **29**, 515-526 (1963).
8. Clayton, R. M. in *The Eye* vol. 5 (eds Davson, H. & Graham, L. T.) 400-494 (Academic, London, 1974).
9. Rabaey, M. *Expl Eye Res.* **1**, 310-316 (1962).
10. Zwaan, J. & Ikeda, A. *Expl Eye Res.* **7**, 301-311 (1968).
11. Genis-Galvez, J. M., Maisel, H. & Castro, J. M. *Expl Eye Res.* **7**, 593-602 (1968).
12. Yoshida, K. & Katoh, A. *Expl Eye Res.* **11**, 184-194 (1971).
13. Piatigorsky, J., Webster, H. deF. & Craig, S. P. *Dev Biol.* **27**, 176-189 (1972).
14. Reszelbach, R., Shinohara, T. & Piatigorsky, J. *Expl Eye Res.* (in the press).
15. Chylack, L. T., Jr & Kinoshita, J. H. *Invest. Ophthalmol.* **8**, 401-412 (1969).
16. Andr  e, G. *Ber. Versamm. dt. ophthalm. Ges.* **70**, 354-358 (1970).
17. Iwata, S. & Kinoshita, J. H. *Invest. Ophthalmol.* **10**, 504-512 (1971).
18. van Heyningen, R. *Expl Eye Res.* **13**, 136-147 (1972).
19. Maraini, G. & Mangili, R. *Ciba Fdn Symp.* **19** (new ser.), 79-97 (1973).
20. Duncan, G. & Bushell, A. R. *Expl Eye Res.* **20**, 223-230 (1975).
21. Chylack, L. T., Jr & Kinoshita, J. H. *Expl Eye Res.* **14**, 58-64 (1972).
22. Chylack, L. T., Jr & Kinoshita, J. H. *Expl Eye Res.* **15**, 61-69 (1973).
23. Lubin, M. & Ennis, H. L. *Biochim. biophys. Acta* **80**, 614-631 (1964).
24. Lubin, M. *Nature* **213**, 451-453 (1967).
25. Carrasco, L. & Smith, A. E. *Nature* **264**, 807-809 (1976).
26. Kuck, J. F. R., Jr. in *Biochemistry of the Eye* (ed. Graymore, C. N.) 183-260 (Academic, London, 1970).
27. Dahl, J. L. & Hokin, L. E. *A. Rev. Biochem.* **43**, 327-356 (1974).
28. Kinoshita, J. H., Kern, H. L. & Merola, L. O. *Biochim. biophys. Acta* **47**, 458-466 (1961).
29. Bonting, S. L., Caravaggio, L. L. & Hawkins, N. M. *Archs Biochem. Biophys.* **101**, 47-55 (1963).
30. Bonting, S. L. *Invest. Ophthalmol.* **4**, 723-738 (1965).
31. Harris, J. E. & Becker, B. *Invest. Ophthalmol.* **4**, 709-722 (1965).
32. Becker, B. & Cotlier, E. *Invest. Ophthalmol.* **1**, 642-645 (1962).
33. Kinsey, V. E. & Reddy, D. V. N. *Invest. Ophthalmol.* **4**, 104-116 (1965).
34. Riley, M. V. *Expl Eye Res.* **9**, 28-37 (1970).
35. Duncan, G. *Ciba Fdn Symp.* **19** (new ser.), 99-116 (1973).
36. Kinsey, V. E. *Eye Structure, II Symp.* (ed. Rohen, J. W.) 383-394 (Schattauer, Stuttgart, 1965).
37. Spector, A. *Invest. Ophthalmol.* **4**, 579-591 (1965).
38. Bloemendal, H. *Science* **197**, 127-138 (1977).
39. Piatigorsky, J., Zelenka, P. & Simpson, R. T. *Expl Eye Res.* **18**, 435-446 (1974).
40. Piatigorsky, J. *J. biol. Chem.* **251**, 4416-4420 (1976).
41. Sonenshein, G. E. & Brawerman, G. *Biochemistry* **15**, 5497-5501 (1976).
42. Saborio, J. L., Pong, Sheng-Shung & Koch, G. *J. molec. Biol.* **85**, 195-211 (1974).
43. Nuss, D. L., Oppermann, H. & Koch, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1258-1262 (1975).
44. Nuss, D. L. & Koch, G. *J. Virol.* **17**, 283-286 (1976).
45. Schochetman, G. & Schlom, J. *Virology* **73**, 431-441 (1976).
46. Lodish, H. *J. biol. Chem.* **246**, 7131-7138 (1971).
47. McKeehan, W. L. *J. biol. Chem.* **249**, 6517-6526 (1974).
48. Sonenshein, G. E. & Brawerman, G. *Biochemistry* **15**, 5501-5506 (1976).
49. Lodish, H. *A. Rev. Biochem.* **45**, 39-72 (1976).
50. Zelenka, P. & Piatigorsky, J. *Expl Eye Res.* **22**, 115-124 (1976).
51. Delcour, J. & Papaconstantinou, J. *Biochem. biophys. Res. Commun.* **57**, 134-141 (1974).

# letters to nature

## Quasar-galaxy pairs and surface density of quasars

DISCOVERIES of radio-quiet quasars in the vicinity of galaxies<sup>1-5</sup>, sometimes with possible signs of interactions seem to contradict strongly the statistical analyses<sup>6-9</sup> on quasar-galaxy associations, which do not support the previous study<sup>10</sup> involving 3C quasars and bright galaxies. In each of these analyses<sup>6-10</sup>, the calculations used the surface densities of galaxies, because the surface density of quasars is poorly known, but it has been claimed<sup>11</sup> that the small angular separations are explicable by chance only if the surface density of quasars is much larger than believed. To test such an assumption we studied the problem by estimating directly the probabilities involved when adopting the most probable values of the density of quasars<sup>12,13</sup> at  $B \sim 19.0-19.5$ , that is,  $\mu \sim 3-5 \text{ (deg)}^{-2}$ .

The formula  $f_1(r) = 2\pi\mu r \exp(-\pi\mu r^2)$  gives the distribution of the angular separations ( $r$ ) between any galaxy and its nearest quasar. The characteristics of this distribution for different values of  $\mu$  are shown in Table 1 with the lower limit of the bilateral 95% confidence level range (column 4). The curves are shown in Fig. 1 with the lower limit taken from column 4 of table 1. For  $\mu \sim 3-5 \text{ (deg)}^{-2}$ , the curves suggest that quasars 3 arc min or more from a galaxy should not be called 'quasars near galaxies' as they occur by chance with more than 2.5% probability. Table 2 lists every radio-quiet quasar within 3 arc min of a galaxy (Arp, ref. 11) (MKN205 is excluded as a compact galaxy<sup>14</sup>). If we assume that Arp's search technique<sup>11</sup> yields quasars of the same nature as the other quasars (there is no evidence to the contrary<sup>15</sup>), for  $\mu = 3$ , nine such pairs would have been found if a search had been made using  $N = 386$  randomly selected quasars ( $P = 2.33\%$ ) and for  $\mu = 5$ ,  $N = 237$  ( $P = 3.80\%$ ). There is then no strong discrepancy with the presently known 140 radio-quiet quasars in the last

Reference Catalogue of Quasars<sup>16</sup>, since only two of these nine were not specifically searched for in the vicinity of galaxies. Moreover, 2 is less than the 3.3 and 5.3 expected pairs for  $\mu = 3$  and 5 respectively with  $N = 140$ .

Some other important effects are (1) two objects in Table 2 are not yet confirmed as quasars.

(2) We need a limiting magnitude for the galaxy, because, for every quasar, we could find a close foreground (or background) galaxy, however faint this galaxy is, if we were not limited by the sky brightness. Then we should exclude cases where very faint galaxies are involved, such as the 18 mag galaxy near 0846 + 51. Moreover, if quasars are associated with bright galaxies, as already suggested, how can they also be found at the distances of the faintest galaxies?

(3) Homogeneous samples of radio-quiet quasars<sup>7</sup> as well as the quasars recently<sup>17,18</sup> detected do not depart from a random distribution with respect to galaxies.

**Table 1** Distribution of the angular distances between quasars and galaxies if quasars are randomly distributed with a mean density  $\mu$

$\mu$ (deg) <sup>-2</sup>	Mean value (arc min)	Variance (arc min) <sup>2</sup>	Lower 95% limit (arc min)
1	29.9	244.0	5.36
2	21.1	122.0	3.79
3	17.4	82.3	3.12
4	14.9	61.0	2.68
5	13.4	49.1	2.41
6	12.3	41.2	2.20
7	11.3	35.2	2.04
8	10.6	30.5	1.90
9	10.0	27.4	1.80
10	9.5	24.6	1.70

**Table 2** Closest radio-quiet quasar-galaxy pairs

Coordinates	Quasar	Galaxy	$m_G$	$z_{QSO}$	$r^*$ (arc min)
1 0151 + 045	PHL1226	IC1746	15.1	0.404	1.8
2 0846 + 512	W1	Anon	~18	†	0.2
3 1108 + 285	QS	NGC3561	14.7	2.192	0.7
4 1132 + 472	BSO1	Anon	15.1	1.13	1.7
5 1222 + 102	Wdm6	NGC4380	13.4	(Cont.)	1.5
6 1233 + 125	Wdm8	NGC4550	12.5	0.728	0.7
7 1344 + 440	BSO1	NGC5296	15.0	0.963	0.9
8 1432 + 489	QS	NGC5682	15.1	1.94	1.7
9 2157 - 133	BSO1	IC1417	~15.0	0.71	1.3

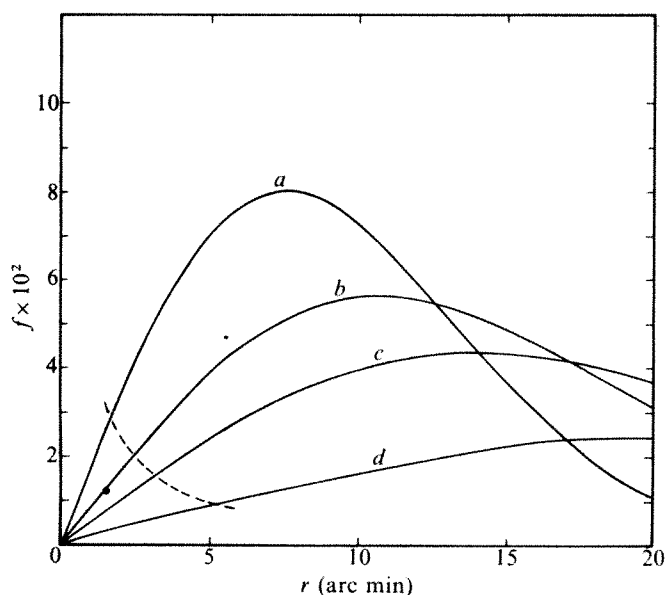
\* $r \leq 3$  arc min, from Arp, ref. 11, except for QSO1108 + 285 (ref. 20).

†Blue variable star-like object.

(4) The choice of the galaxy may introduce two more observational selection effects in these small probabilities. Such probabilities are, in fact, the product of  $P_1 \times P_2$ ,  $P_1$  being the probability of finding a variable or blue stellar object in a particular location with respect to a galaxy<sup>19</sup>, and  $P_2$  the probability that this object is a quasar, equal to the number of pairs over the number of galaxies investigated. As the first step corresponding to  $P_1$  is omitted,  $P_2$  (a few per cent, according to ref. 11) is wrongly compared with the very small (but *a posteriori*) probability by chance ( $\sim 10^{-3}$ ) for such configurations, to prove the unlikelihood of the association; unfortunately the number of galaxies not investigated because of the absence of variable or blue stellar objects is inaccessible. A possible answer is that 'associated' quasars are associated only with galaxies having well-defined characteristics (morphological type, for example). Then the number of pairs involved here is much smaller and the existence of the excluded pairs can only be explained by chance, which proves then that close associations are not as rare as believed.

If a quasar lies within  $r$  arc min of a bright galaxy in the vicinity of which other fainter galaxies lie, (in a group for example, with a density  $\mu'$ ), then in  $P = 0.61 \mu' r^2$  of the cases, there will be another galaxy lying within  $r$  arc min of this galaxy and located nearer the quasar than the parent galaxy ( $P = 8.5\%$  if  $r = 10$  arc min and  $\mu' = 5(\text{deg}^{-2})$ ) and in  $P' = 1 - \exp(-\pi \mu' r^2)$  cases, another galaxy will lie within  $r$  arc min of the quasar ( $P' = 35\%$  with  $r$  and  $\mu'$  as above). In such cases the final angular separation will be

**Fig. 1** Distribution curves of the angular separations for different values of the density of quasars in  $(\text{deg}^{-2})$ . The dashed line indicates the locus of the lower limits of the 95% bilateral level range (see Table 1). a,  $\mu = 10$ ; b,  $\mu = 5$ ; c,  $\mu = 3$ ; d,  $\mu = 1$ .



calculated from the fainter galaxy instead of the bright one around which the search was made (cases of quasars associated with companion galaxies<sup>11</sup>). Such situations, which are not very rare according to the indicative probabilities found here, can artificially increase the number of close associations and reduce the angular distances in the same way.

It is difficult to evaluate quantitatively the result of such observational selection effects, except by a careful systematic search for radio-quiet quasars around galaxies. But if we take these effects into account, as well as the estimated density of quasars and the expected distribution of angular distances, the list of very close quasar-galaxy pairs and the values of the probabilities are not as impressive as before. So far as we can conclude, the small angular distances found between some radio-quiet quasars and galaxies do not disagree with the expected values corresponding to the most likely values of the surface density of quasars. These spectacular associations correspond only to the toes of the distribution of the angular separations and the discovery of such quasars seems to be due only to their exceptional location.

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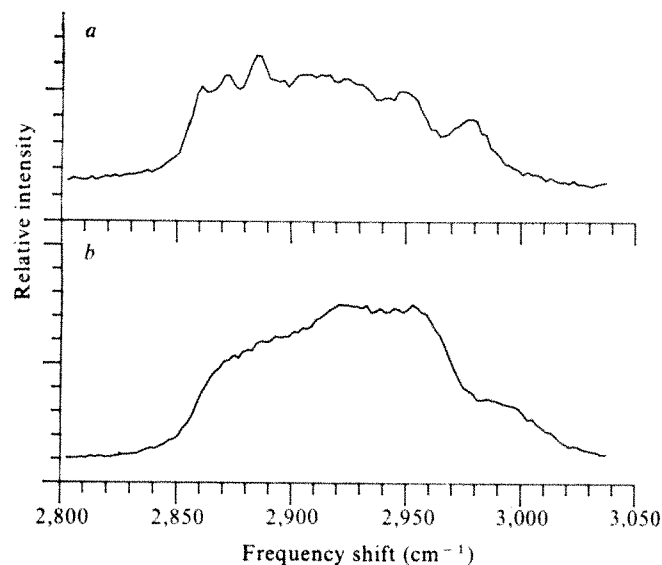
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1. Arp, H. C., Baldwin, J. A. & Wampler, E. J. *Astrophys. J.* **198**, L3-L5 (1975).
2. Arp, H. C., Willis, A. G. & de Ruiter, H. R. *IAU Circ. No.* 2750 (1975).
3. Arp, H. C., Pratt, N. & Sulentic, J. W. *Astrophys. J.* **199**, 565-585 (1975).
4. Arp, H. C., Sulentic, J. W., Willis, A. G. & de Ruiter, H. R. *Astrophys. J.* **207**, L13-L16 (1976).
5. Arp, H. C. *Astrophys. J.* **210**, L59-L61 (1976).
6. Burbidge, G. R., O'Dell, S. L. & Strittmatter, P. A. *Astrophys. J.* **175**, 601-611 (1972).
7. Hazard, C. & Sanitt, N. *Astrophys. Lett.* **11**, 77-82 (1972).
8. Bahcall, J. N., McKee, C. F. & Bahcall, N. A. *Astrophys. Lett.* **10**, 147-152 (1972).
9. Browne, I. W. A. & McEwan, N. J. *Mon. Not. R. astr. Soc.* **162**, 21P-24P (1973).
10. Burbidge, E. M., Burbidge, G. R., Solomon, P. M. & Strittmatter, P. A. *Astrophys. J.* **170**, 233-240 (1971).
11. Arp, H. C. *IAU Colloq.* 37; *C.N.R.S. Colloq.* 263 (1976).
12. Sandage, A. R. & Luyten, W. J. *Astrophys. J.* **155**, 913-918 (1969).
13. Bolton, J. G., Peterson, B. A., Wills, B. J. & Wills, D. *Astrophys. J.* **210**, L1-L3 (1976).
14. Walker, M. F., Pike, C. D. & Wills, B. J. & Wills, D. *Astrophys. J.* **210**, L1-L3 (1976).
15. Nieto, J.-L. thesis, Univ. Paris (1976).
16. Burbidge, G. R., Crowne, A. H. & Smith, H. E. *Astrophys. J. Suppl.* **33**, 113-188 (1977).
17. Bond, H. E., Kron, R. G. & Spinrad, H. *Astrophys. J.* **213**, 1-7 (1977).
18. Osmer, P. S. & Smith, M. G. *Astrophys. J.* **213**, 607-618 (1977).
19. Weedman, D. W. *Astrophys. Lett.* **9**, 49-51 (1971).
20. Stockton, A. *Astrophys. J.* **155**, L141-L142 (1969).

## Pressure-induced changes in molecular conformation in liquid alkanes

LIQUID linear alkanes at room temperature and pressure exist in a mixture of molecular shapes. The *n*-heptane molecule, for example, has 13 possible distinct conformations with up to four gauche bonds distributed along its length. In the crystal, however, all the molecules assume the all-*trans*, straight chain shape. We have studied the conformations of chain molecules in the liquid state in various conditions using Raman scattering as a probe<sup>1</sup>. We have looked particularly at what happens to the shapes of linear alkane molecules subjected to high pressures in the liquid state. We speculated that as the pressure on a liquid of chain molecules was increased toward the freezing point that the chains might begin to straighten out. In fact, we found that an increase in pressure caused an increase in the number of gauche bonds, that is, pressure caused the molecules to become more globular.

Our interest in this investigation grew out of some recent discoveries concerning the conformation of linear polyethylene, a very long-chain molecule whose length can be as much as 1  $\mu\text{m}$  or more. Typically, polyethylene crystallised from the melt folds itself into straight chain segments tens of nanometres long, arranged side-by-side to form lamellae, with the segment axes perpendicular to the lamellar surface. The resulting material has relatively poor mechanical strength, presumably because

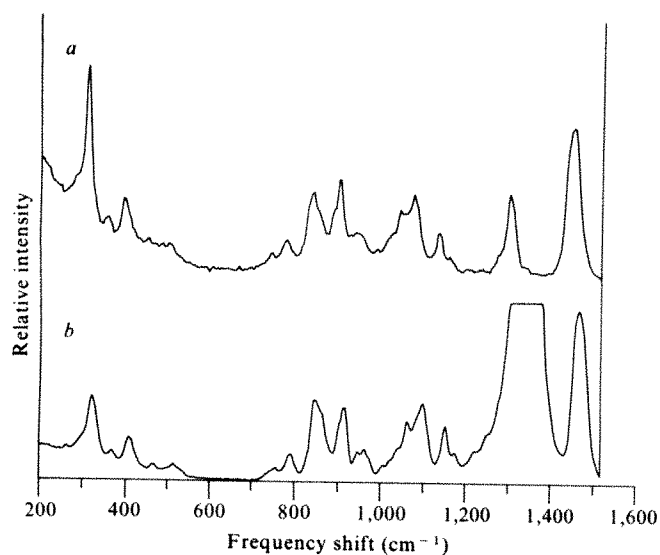


**Fig. 1** The CH stretching bands for heptane at 65 °C and pressures of *a*, 1 atm and *b*, 14.7 kbar. The peak at 2,880 cm<sup>-1</sup> disappears at high pressure as the molecules become more globular in shape.

it is held together mainly by Van der Waals forces between lamellae rather than by the carbon-carbon bonds of the polyethylene chain. On the other hand when polyethylene is crystallised at high pressure, ~ 5 kbar, the chains are extended to their full lengths in the solid<sup>2</sup> and, still more interesting, there is evidence that in the high pressure liquid before crystallisation the polyethylene chains exist in a 'nematic'-like state, an ordered liquid phase<sup>3</sup>. We wished to investigate this ordered phase and because the spectroscopy was more straightforward, decided to begin with short chain alkanes. Our results indicate that for the short chains the ordered liquid phase does not exist.

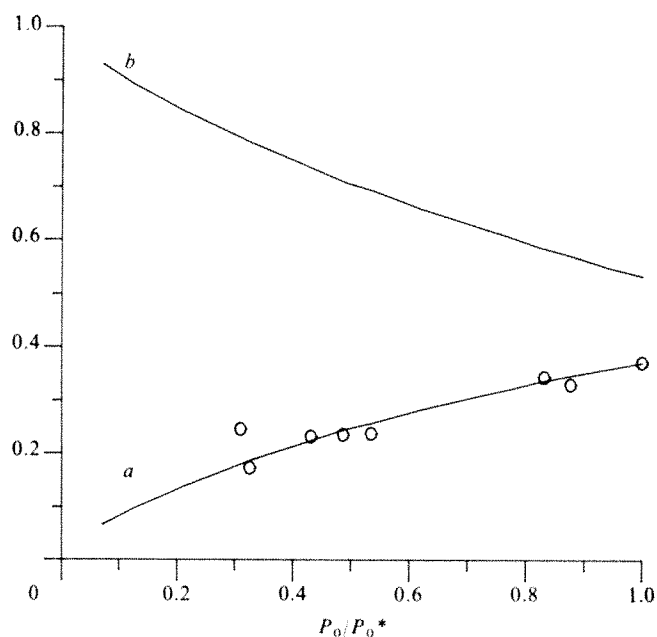
For heptane at room temperature and pressure the relative population of the all-*trans* conformer is about 15%<sup>4</sup>. The populations of other conformers can be calculated using the Boltzmann distribution, with each *gauche* bond requiring ~ 500 cal mol<sup>-1</sup> of energy. The greater statistical weight of the

**Fig. 2** Spectra of heptane as in Fig. 1 but for the lower frequency range. The intensities of the acoustic bands in the region 300–600 cm<sup>-1</sup> drop as the pressure rises, indicating that the populations of molecules in the all-*trans* conformation and molecules with one *gauche* bond are decreasing in favour of more highly kinked conformers.



*gauche* conformers overcomes the energy barrier and for liquid alkanes longer than nonane the *trans* population is small and spectroscopic observation of the *trans* species is extremely difficult.

In the Raman spectrum of the alkanes there are two frequency domains that are especially conformation sensitive: the high frequency C-H stretching bands and the low frequency acoustic bands. The high frequency region for heptane at 65 °C is shown in Fig. 1. The upper spectrum was taken at room pressure and shows a fairly sharp peak near 2,880 cm<sup>-1</sup>, the intensity and



**Fig. 3** Relative populations of molecules with one *gauche* bond, *a*,  $P_1$ , and with more than one *gauche* bond, *b*,  $P_{2-4}$ . The abscissa is the all-*trans* population,  $P_0$ , relative to its room pressure value,  $P_0^*$ . Pressure is increasing to the left therefore. Solid curves are theoretical predictions; open circles are data points.

sharpness of which has been shown to be proportional to the crystallinity and packing density of neighbouring chain molecules<sup>5</sup>. In the lower curve taken in the liquid at 14.7 kbar pressure this band has disappeared, indicating a probable reduction in lateral chain order.

Figure 2 shows the spectral range 200–1,500 cm<sup>-1</sup> for the same two pressures for heptane. Most of this spectral range is only slightly affected by the change in pressure and the band at 1,450 cm<sup>-1</sup> was used as an intensity standard. (The pressures were generated in a diamond anvil cell. The strong feature appearing in the high pressure spectrum near 1,300 cm<sup>-1</sup> is the diamond Raman band.) The frequency range 300–600 cm<sup>-1</sup> contains the acoustic modes which are affected quite strongly by pressure. These bands have been the subject of much study<sup>6,7</sup>. Model calculations have been made, and some of the normal modes for different conformations have been identified with these bands<sup>8</sup>.

The sharp peak near 309 cm<sup>-1</sup> is the so called 'longitudinal acoustic mode' (LAM) which is due to a vibration of the all-*trans*, TTTT, conformers along their chain axes. Its frequency is inversely proportional to the chain length. The band at 507 cm<sup>-1</sup> results from a molecule with a single *gauche* bond, TGTT, the band at 363 cm<sup>-1</sup> is due to the GTTT conformer, and the band at 396 cm<sup>-1</sup> is caused by a superposition of bands from a single *gauche* bond conformation, GTTT, and a double *gauche* conformer, TTGG.

Note that in the room pressure spectrum the all-*trans* band at 309 cm<sup>-1</sup> is by far the strongest peak although it accounts for only 15% of the molecular population. It seems



that as the molecules become more globular in shape, vibrations along the chain length have less effect on the polarisability. The intensities of bands for 3- and 4-*gauche* bond conformers are too weak to be detected.

It is apparent from Fig. 2b that all the bands in the low-frequency region decrease as the pressure increases with the LAM dropping more rapidly than its neighbours. This shows that the populations  $P_0$ , of the all-*trans* molecules, and  $P_1$ , of the single-*gauche* bond molecules, are decreasing in favour of  $P_{2-4}$ , the population of the 2-, 3-, and 4-*gauche* bond conformers.

We have developed a theory, generalised from liquid-crystal theory<sup>9</sup>, which predicts this behaviour. Our model consists of a dense gas of hard rods interacting only through excluded volume effects and Van der Waals attraction. The rods have no orientational correlations and are free to rotate end over end as in the case of an isotropic liquid crystal. Different conformers of heptane correspond to different species of rods, each with its own aspect ratio (length to breadth ratio) and energy (depending upon the number of *gauche* bonds). If the aspect ratio of a rod decreases, the excluded volume swept out as it tumbles is reduced and the translational entropy increases. If we calculate and minimise the Gibbs free energy for this model we find that rods with lower aspect ratios become more favoured as the pressure and density increase. This means that the molecules become more globular as the pressure rises.

Figure 3 shows the predictions for the rise of  $P_{2-4}$  and the drop of  $P_2$  as functions of  $P_0/P_0^*$  ( $P_0^*$  is the room pressure value of  $P_0$ ). Pressure therefore is increasing to the left in this figure. The agreement between theory and experiment is gratifying.

We have also investigated the conformational behaviour of hexane, octane, and hexadecane. The first two have behaviour similar to that of heptane. We recall that there is evidence for an ordered phase in a polyethylene liquid at high pressure. Since such a phase does not seem to exist in the short chain liquids one might expect to find a 'crossover' chain length above which the ordered phase could be found. Hexadecane at room pressure is made up almost entirely of *gauche* bonded conformers. We found that as the pressure was increased no significant change in the conformational population could be detected spectroscopically up to the freezing point for temperatures up to 165 °C. We conclude that either hexadecane is shorter than the 'crossover' length or that the ordered phase is inaccessible in this temperature range. We are continuing our investigation with other alkanes and polyethylene.

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1. Schoen, P. E., Schnur, J. M. & Sheridan, J. P. *Appl. Spectrosc.* **31**, 337 (1977).
2. Wunderlich, B. & Arakawa, T. *J. Polymer Sci.* **A2**, 3697 (1964).
3. Monobe, K., Fujiwara, Y. & Tanaka, I. *Proc. Conf. High Pressure* 63 and 865 (Kawakita Kyoto, 1975).
4. Bartell, L. S. & Kohl, D. A. *J. chem. Phys.* **39**, 3097 (1963).
5. Gaber, B. P. & Petricolas, W. L. *Biochim. biophys. Acta* **465**, 260 (1977).
6. Snyder, R. G. & Schachtschneider, J. H. *Spectrochim. Acta* **19**, 85 (1963).
7. Snyder, R. G. *J. chem. Phys.* **47**, 1316 (1967).
8. Schaufele, R. F. *J. chem. Phys.* **49**, 4168 (1968).
9. Alben, R. *Molec. Cryst. Liq. Cryst.* **13**, 193 (1971).

## Particle size analysis for machinery health monitoring

MONITORING the health, or condition, of machinery is a universally accepted way of achieving economy of use and preventing catastrophic failure. Wear debris analysis is a powerful method of monitoring oil-lubricated machinery and many techniques are used, such as spectroscopic oil-analysis<sup>1</sup>, magnetic plug and filter inspection<sup>2</sup>, ferrography<sup>3</sup>, and particle size analysis. Although this

Table 1 Particle size analysis

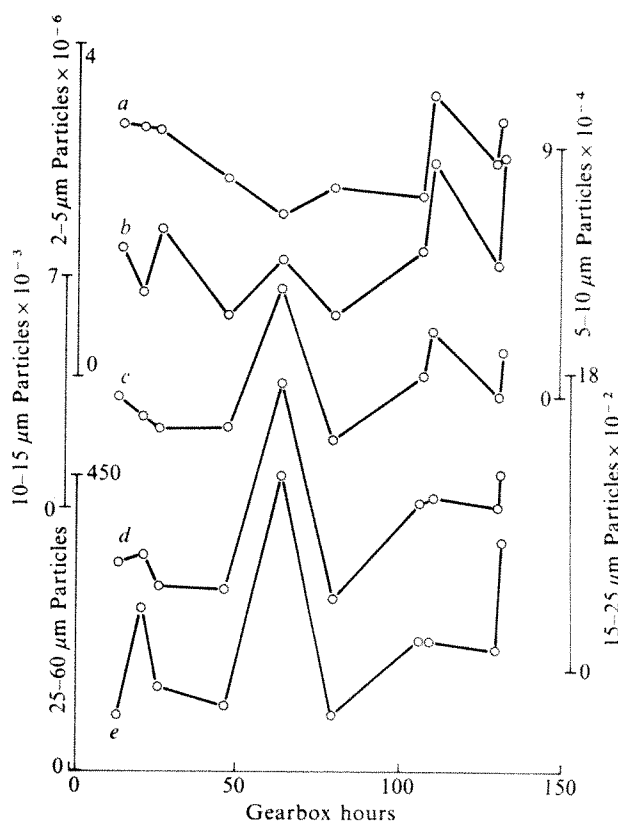
Sample no.	Gearbox hours	$n$	$b$ ( $\mu\text{m}$ )	$r$	Iron content ( $\text{mg l}^{-1}$ )
1	15	3.183	0.907	0.955	7.0
2	22	2.514	0.540	1.000	7.1
3	26.75	3.068	0.842	1.000	7.9
4	46.75	2.830	0.682	0.996	5.5
5	63.25	2.382	0.725	0.994	5.5
6	79.50	2.900	0.707	0.998	4.6
7	106.75	2.773	0.843	0.997	5.0
8	109.25	3.024	0.935	0.998	8.8
9	129.75	2.744	0.745	0.997	6.9
10	130.25	2.755	0.830	1.000	7.4

These values of  $n$ ,  $b$  and  $r$  were obtained with a lower size limit,  $d'$ , of 2  $\mu\text{m}$ ; this value corresponds to the limit of the sensor used.

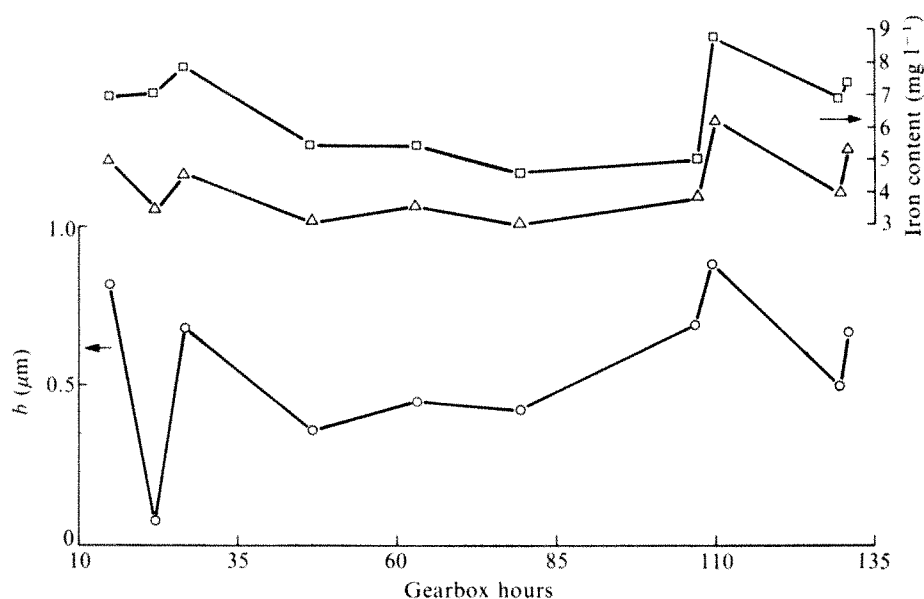
last technique is frequently used for monitoring hydraulic fluids, where contamination levels are low, it has not been accepted as a means of monitoring relatively dirty systems such as gas turbines where other techniques are more efficient and easier to interpret. Here we show how the results from particle size analysis can be reduced to a simple indicator of the severity of wear; this indicator is related to spectroscopic oil analysis and shows promise as a method of monitoring the new generation of ultra-clean gas turbines.

The cumulative distribution of wear particles as a function of particle size can be fitted to the distribution function,  $P(d) = \exp - [b/(d-d')]^n$ ;  $P(d)$  is the probability of a particle being smaller than  $d$ ,  $d'$  is a lower particle size limit,  $b$  and  $n$  are the independent parameters which may be adjusted to fit the experimental results. This function is similar to that used by Rosin and Rammler<sup>4</sup> to describe the fineness of powdered coal. Taking logarithms twice

Fig. 1 Particle counts per 100 ml of oil for a, 2–5  $\mu\text{m}$ ; b, 5–10  $\mu\text{m}$ ; c, 10–15  $\mu\text{m}$ ; d, 15–25  $\mu\text{m}$ ; e, 25–60  $\mu\text{m}$ . The apparatus used works on the principle of light blockage in which particles passing through a cell interrupt a light beam incident on a photo-diode. As the particle passes through the cell it tumbles and the maximum projected area is detected and the particle is sized as having the diameter of a sphere with the same cross-sectional area.



**Fig. 2** Values of  $b$  (○), measured iron content (□), and calculated iron content (△) as a function of gearbox hours. The calculated iron contents were determined from the integral  $T\rho\int_0^{20}\pi d^3/24p(d)dd$  where  $p(d) = d P(d)/dd$ ,  $T$  is the total number of particles per litre and  $\rho$  is the density of steel. The integral was evaluated using Simpson's rule.



yields  $\ln[\ln(1/P(d))] = -n\ln(d-d') + n\ln b$ . Thus a plot of  $\ln[\ln(1/P(d))]$  against  $-\ln(d-d')$  should be linear if the distribution function is applicable to the experimental results. From the form of  $P(d)$  we see that 36.7% of the particles will be smaller than  $b+d'$ . Since it is generally accepted that an increase in the relative number of large particles is an indication of an increasing severity of wear, it may be possible to use  $b$  as an indicator of the severity of wear.

Ten oil samples from a helicopter gearbox test-rig were used to test this theory. The particle sizes were analysed with a 'Hi-Ac' automatic particle size analyser; the iron contents were measured using an X-ray fluorescence spectrometer. The particle size analysis for the 10 samples is shown in Fig. 1. The values of  $n$ ,  $b$  and  $r$  (the regression coefficient) obtained by fitting the particle counts to the distribution function, and the measured iron contents are given in Table 1.

Figure 2 compares the values of  $b$ , the measured iron contents and the calculated iron contents. The close agreement between the trends in  $b$  and the measured iron content is striking and shows that  $b$  can be used as an indicator of wear. A comparison of Figs 1 and 2 illustrates how this analysis has simplified the somewhat complicated results of particle counting.

Figure 2 also shows the good agreement between the trends of the measured and calculated iron contents. Since wear particles are rarely spherical the expression for the iron content would be expected to be an overestimation. The good agreement actually observed is presumably due to the fact that particles smaller than  $2 \mu\text{m}$  are ignored by the particle counter.

I believe that this new interpretation of particle size analysis may well become an important technique for machinery health monitoring, especially as the trend towards improved filtration reduces the relative sensitivity of spectroscopic oil analysis.

Further details of this analysis as well as the relationship between particle size analysis and direct reading ferrography will be published elsewhere. I thank Mr P. Gadd of the Naval Aircraft Materials Laboratory for the particle size analysis.

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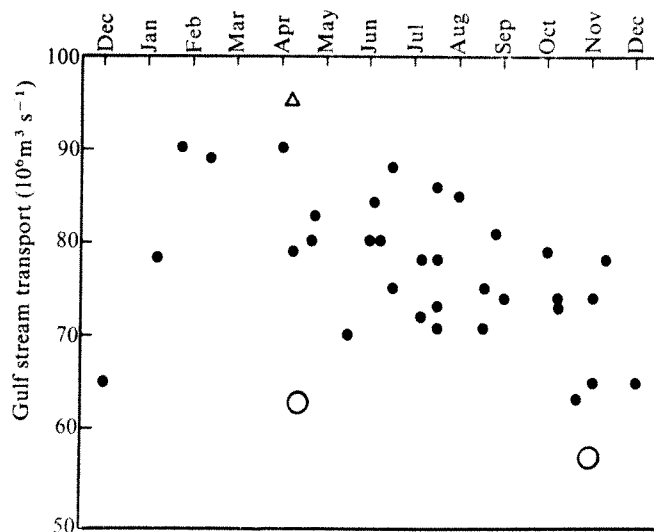
Received 12 August; accepted 13 October 1977.

1. Bond, A. & Kittinger, D. C. *Proc. Ann. natn. Aerospace Electronics Conf.* Dayton 251-256 (1966).
2. Hunter, R. C. *Aircraft Engng* 4-14 (1975).
3. Bowen, R., Scott, D., Seifert, W. & Westcott, V. C. *Tribology Int.* 109-115 (1976).
4. Rosin, P. & Rammler, E. J. *Inst. Fuel* 7, 29-36 (1933).

## Intensification of the Gulf Stream after the winter of 1976-77

THE eastward volume transport of the Gulf Stream south of New England varies within a wide range. Within a triangle whose apex is at Bermuda and whose base extends from Cape Hatteras to Cape Sable, Nova Scotia, there have been 32 reliable oceanographic sections made across the Stream between 1932 and 1968. Geostrophic volume transports for these sections, computed relative to 2,000 m, are shown in Fig. 1. This 2,000 m surface is necessary if any seasonal change in transport is to be detected; only 10 of these sections reach to the ocean bottom ( $\sim 4,500 \text{ m}$ ), and these were all made between April and August. These results tend to confirm an earlier suggestion<sup>1</sup> that the Gulf Stream is strongest in late winter and weakest in late autumn; this was attributed to the strengthening of the zonal winds in the winter. More recently, I have postulated a different mechanism for this annual variation in transport. The two most intense ocean currents, the Gulf Stream and the Kuroshio, are found on the western side of northern hemisphere oceans. These oceanic regions are visited throughout the winter by frequent outbreaks of frigid polar continental air. As a result of these outbreaks, very deep mixed layers are formed immediately south of these currents in late winter. The greatest thermocline depths are always found directly beneath the most deeply mixed layers, and it is primarily the variations in thermocline depth which account for the variations in computed transport shown in Fig. 1; the deeper the thermocline south of the Stream, the larger the transport. I suggest that the Gulf Stream receives a new charge of energy at the end of each winter due to this deepening of the thermocline. Here I discuss how the cold winter of 1976-77 influenced the Gulf Stream transport.

The temperature distribution in the western North Atlantic can be seen in the first more-or-less meridional section across the Sargasso Sea made by Thomson<sup>2</sup> in HMS Challenger. This section (Fig. 2) passed across the Gulf Stream in May; spring warming had started up at the sea surface above the deep, nearly mixed layer immediately south of the Gulf Stream. The Gulf Stream System is an asymmetric anticyclone. The narrow, intense eastward flow of the Gulf Stream itself takes place between



**Fig. 1** Computed geostrophic volume transports for sections in the Bermuda - Nova Scotia - Cape Hatteras triangle: ●, 32 sections made between 1932 and 1968; ○, sections made before and after the winter 1974-75; △, between Researcher stations 6 and 33 in April 1977. All transports are relative to 2,000m and are plotted against month.

Challenger stations 42 and 44. The slower and much wider return flow toward the west takes place between stations 42 and 24, augmented by a further westward flow through the Caribbean Sea south of station 24. The maximum top-to-bottom transport of the anticyclone has been estimated to be about  $150 \times 10^6 \text{ m}^3 \text{ s}^{-1}$  (refs 4, 5) of which only about  $30 \times 10^6 \text{ m}^3 \text{ s}^{-1}$  pass through the Caribbean. Tropical surface water from the Caribbean can be seen as the 'warm core' at Challenger station 43. Superimposed on the anticyclone one can infer a shallow, meridional circulation in which excess  $18^\circ \text{C}$  water (ref. 6), formed at the end of the winter, flows south at the 300 m level and surface water is advected north to replace it. This process does not directly affect the Gulf Stream transport.

It can be seen (Fig. 2) that the thermocline is, in fact, deepest in the centre of the anticyclone beneath the thickest part of the lens of  $18^\circ \text{C}$  water. To the north, the thermocline rises abruptly across the Gulf Stream, and the lens of  $18^\circ \text{C}$  water disappears abruptly. To the south the thermocline rises gradually and the lens of  $18^\circ \text{C}$  attenuates gradually.

It is difficult to test hypotheses in the ocean, but an attempt was made during 1974-75 to observe the formation

of  $18^\circ \text{C}$  water and its possible effect on the anticyclone. An extensive oceanographic survey was made in October and November of 1974 and repeated in March 1975. No deep mixed layers were formed, and the  $18^\circ \text{C}$  water was not renewed by the atmosphere. Research ship cruises must be planned a year or two ahead of time, hence observers interested in short-scale climatic changes must simply take their chances. Precise calculation of the heat flux from ocean to atmosphere cannot be made at present since there is a three or four year lag before the necessary marine meteorological data can be obtained on magnetic tape, but the winter of 1974-75 was exceptionally mild over the northwestern Sargasso Sea.

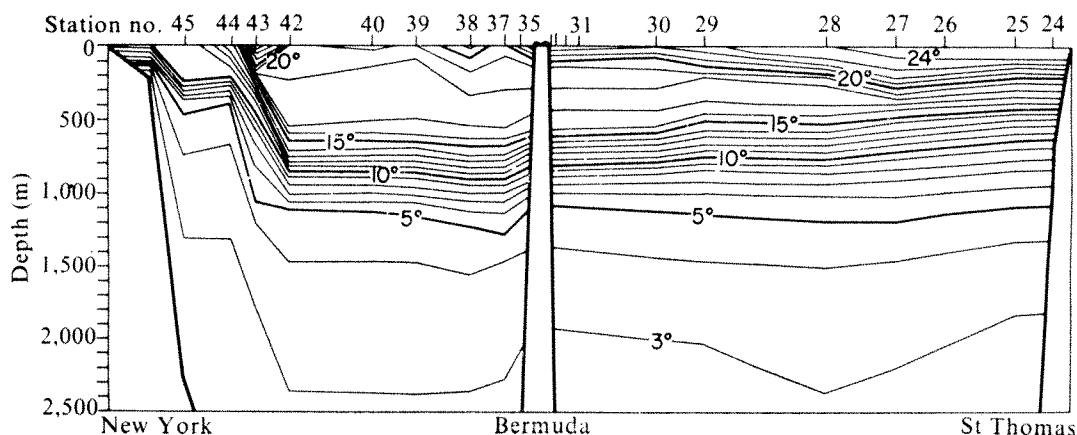
The winter of 1976-77, however, has been exceptionally cold in the eastern US. The surface atmospheric pressure map for mid-January to mid-February 1977 is drawn in Fig. 3. It is marked by a shift of the Icelandic low towards Newfoundland and a retreat of the Azores-Bermuda high towards the east. This was accompanied by a flow of continental polar air across the northwestern Sargasso Sea. This pattern was present much of the winter but broke up completely in March 1977.

I was invited aboard the Ocean Survey ship Researcher in April 1977 to investigate the effect of this remarkable winter on the  $18^\circ \text{C}$  water and its influence, if any, on the Gulf Stream transport.

Again, precise heat flux computations cannot be made at present, but the contrast between the mixed layer depths after the mild winter and the severe winter was vivid. Late winter temperature-depth and dissolved oxygen-depth curves for neighbouring oceanographic stations near the southern edge of the Gulf Stream are shown in Fig. 4. Knorr station 577 ( $34^\circ 31' \text{N}$ ,  $72^\circ 31' \text{W}$ ; 10 March 1975) has a mixed layer only 175 m deep. A faint inflexion can be seen in the  $18^\circ \text{C}$  water at 300 m. This is probably a relic of a previous winter when renewal of  $18^\circ \text{C}$  water by the atmosphere did take place. The concentration of dissolved oxygen at 300 m was 4.45 ml per l (81% of saturation), indicating that biological consumption of oxygen had been going on for some time. Below this inflection the temperature gradient increases rapidly to  $1^\circ \text{C}$  per 50 m, the normal value for the thermocline in the Sargasso Sea. The depth of the  $10^\circ \text{C}$  isotherm, a commonly used index of thermocline depth, is 815 m.

The upper 200 m of Researcher station 33 ( $34^\circ 30' \text{N}$ ,  $71^\circ 18' \text{W}$ ; 12 April 1977) is composed of 'warm core' Gulf Stream water, but the rest of the top 600 m consists of newly formed  $18^\circ \text{C}$  water with oxygen values of about 5.1 ml per l (96% of saturation). The thermocline is much deeper: the  $10^\circ \text{C}$  isotherm lies at a depth of 1,020 m,

**Fig. 2** Temperature section from St Thomas, Virgin Islands, to Bermuda to New York, made from HMS Challenger in spring, 1873.



deeper than at any of the earlier stations used for the computation of Gulf Stream transport (Fig. 1).

Computed volume transports for the two sections made before and after the mild winter of 1974–75 are also plotted in Fig. 1. While the late winter section ( $62 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ ) is slightly higher than the autumn section ( $57 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ ), these two sections give the two least volume transport figures ever computed, relative to the 2,000 m surface, in this area. Researcher station 33 has been paired with the nearest station north of the Gulf Stream (Researcher station 6;  $37^\circ 46' \text{N}$ ,  $72^\circ 00' \text{W}$ , 1 April 1977), and the computed volume transport between them,  $95 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ , has also been plotted in Fig. 1. It is the largest volume transport ever computed for the Gulf Stream.

With reservations, these data add credence to my proposed mechanism of oceanic anticyclogenesis<sup>2</sup>. The

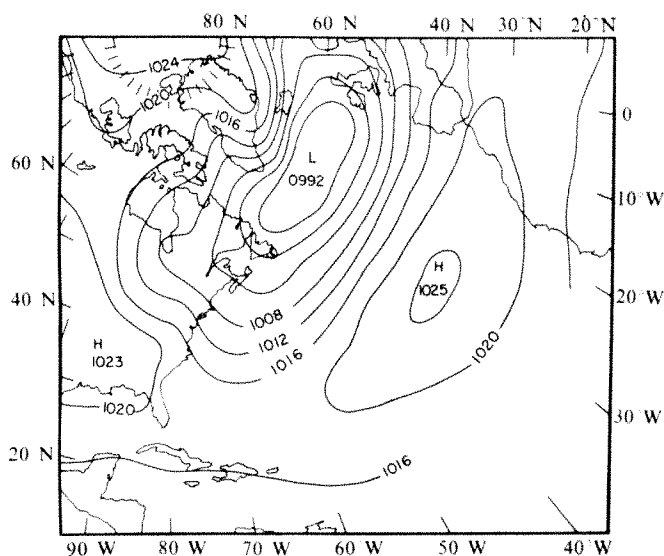


Fig. 3 Average surface atmospheric pressure for the period mid-January to mid-February 1977.

main reservations are these: Researcher was equipped with expendable bathythermographs that recorded the temperature down to 760 m. Thus it was possible to place station 33 in the exact centre of the region where the mixed layer was deepest and the thermocline was deepest. Knorr was similarly equipped during the two cruises which showed the lowest volume transports (Fig. 1) and these low figures are undoubtedly true. These sections were made, however, in a different longitude ( $64^\circ 30' \text{W}$ ) on the Nova Scotia side of the Hatteras–Bermuda–Nova Scotia triangle where the current (in the upper layers) may be weaker<sup>5</sup>. Also, the areas where the deepest mixed layers were found were not very wide. Such deep mixed layers could easily have been missed by earlier observers. It is further possible to assume that Researcher station 33 was in the centre of a small, local anticyclone independent of the Gulf Stream. Nevertheless, it seems that the Gulf Stream gained strength after the cold winter of 1976–77. I attribute this to deep winter overturn. It is hard to see how this intensification can have been due to wind-stress alone in view of the air-flow pattern indicated by surface atmospheric pressure (Fig. 3). The theoretical Gulf Stream transport corresponding to the mean wind-stress field is only  $23 \times 10^6 \text{ m}^3 \text{ s}^{-1}$  (see ref. 7). Thus the gross variability in baroclinic transport in the upper half of the Gulf Stream ( $57$ – $95 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ ) is nearly double the total mean theoretical wind-driven transport.

Masuzawa<sup>8</sup> has shown a meridional section across the Kuroshio system made in January 1967 in which deep

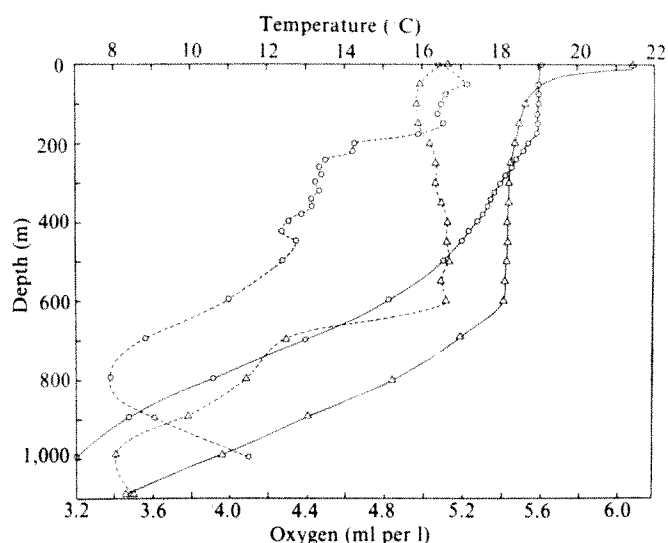


Fig. 4 Temperature–depth (solid lines) and oxygen–depth (dashed lines) curves for stations made south of the Gulf Stream in late winter 1975 (○) and early spring 1977 (△).

mixing may have contributed to the depression of the thermocline south of Japan. This section also shows the equatorward spreading of subtropical mode water, the northwest Pacific analogue of  $18^\circ \text{C}$  water. McCartney<sup>9</sup> has discovered that a similar process takes place on the equatorward side of the Antarctic Circumpolar Current. It seems that the greatest current systems in the world oceans are accompanied by deep mixing on their equatorward sides, and it is possible that they derive the greater part of their energy from this process.

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1. Iselin, C.O'D. *Pap. Phys. Oceanogr. Met.*, **8**, No. 1, 1–40 (1940).
2. Worthington, L. V. In *Studies in Physical Oceanography—a tribute to Georg Wüst on his 80th birthday* (ed. Gordon, A. L.) **1**, 169–178 (Gordon and Breach, New York, 1972).
3. Thomson, C. W. in *The Atlantic*, **1**, 1–424 (Macmillan, London, 1877).
4. Fuglister, F. C. *Prog. Oceanogr.*, **1**, 265–373 (Pergamon, London, 1963).
5. Worthington, L. V. *The Johns Hopkins Oceanographic Studies* no. 6, 110 (The Johns Hopkins University Press, Baltimore, 1976).
6. Worthington, L. V. *Deep-Sea Res.*, **5**, 297–305 (1959).
7. Welander, P. in *The Atmosphere and the Sea in Motion* (ed. Bolin, B. B.) 95–101 (Rockefeller Institute Press and Oxford University Press, New York, 1959).
8. Masuzawa, J. in *Kuroshio: Its Physical Aspects* (eds. Stommel, H. & Yoshida, K.) 95–127 (University of Tokyo Press, Tokyo, 1972).
9. McCartney, M. S. *Deep-Sea Res. Suppl.* **24** (in the press).

## Manganese-rich particulate matter in a coastal marine environment

PARTICULATE matter in the waters of the Gulf of St Lawrence, a major marginal sea, contains as much as 16,500 p.p.m. manganese. This is comparable with the levels of manganese found in deep-sea sediments<sup>1</sup>. Here we show that it is possibly a related phenomenon. Suspended particulate matter was collected by filtration on  $0.4\text{-}\mu\text{m}$  Nucleopore filters on a series of six stations along the axis of the Laurentian Trough. The samples were digested in a Teflon bomb with a mixture of aqua regia and hydrofluoric acid, and analysed by atomic absorption spectrophotometry<sup>2</sup>. Figure 1 shows a typical vertical profile of the concentration of suspended particulate matter and its content of manganese. The manganese content increases



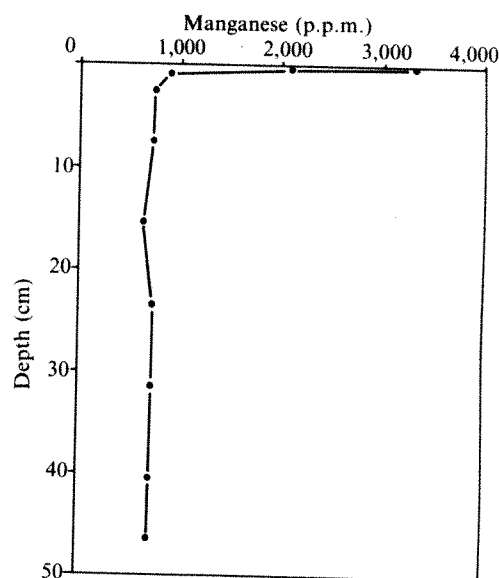
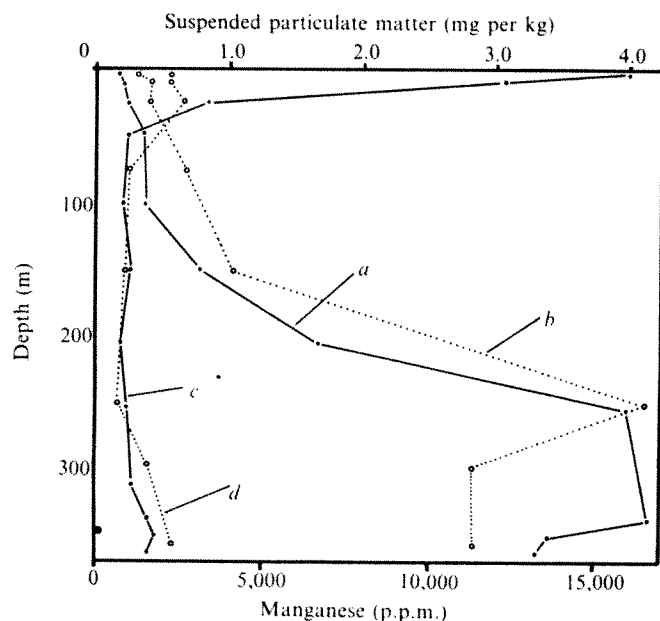
rapidly with depth to a maximum value of 16,500 p.p.m. at 40–120 m above the bottom. The profiles are similar for the two cruises.

Resuspension of manganese-rich bottom sediment, and diffusion of dissolved manganese out of the sediment with subsequent precipitation in the water column, are two mechanisms which may produce manganese-rich particulate matter. Figure 2 shows that a manganese-rich surface layer, only a few millimetres thick, covers a sediment which otherwise is uniformly low in manganese. Higher concentrations of particulate matter near the bottom than at intermediate depths exist throughout the deep parts of the Gulf of St Lawrence<sup>3</sup>, indicating that resuspension of bottom sediments is an active mechanism<sup>4</sup>. But, the manganese content of the near-bottom particulate matter is higher than in the upper 1 mm of the sediment (by a factor of 4 to 5). Resuspension as the sole responsible mechanism requires, therefore, that the particulate matter retained in suspension—that is the slow settling components of the resuspended sediment—contain much more manganese than the bulk sediment. This possibility cannot be confirmed, however, because there are no data available on the manganese content of these sediments as function of settling rates.

The thinness of the manganese-rich sediment layer, and by inference the oxidised zone, indicates that manganese dissolved in the pore water would only have to diffuse a short distance to pass from the reduced zone to the water column. Subsequent precipitation would then result in manganese-rich particulate matter; this mechanism has been proposed by Evans *et al.*<sup>5</sup>. Graham *et al.*<sup>6</sup> have shown that diffusion of dissolved manganese out of sediments is fast enough to be a major factor in the manganese balance in Narragansett Bay.

Some of the manganese-rich particulate matter may reach the seaward-moving layer of the water-column by vertical mixing and advection, and ultimately find its way to the open ocean. This is supported by the data of Bewers and Yeats<sup>7</sup>, which show that whereas 93% of the input of particulate matter to the Gulf of St Lawrence sediments internally, only 47% of the manganese input is lost in this

**Fig. 1** Vertical distribution of the concentration and manganese content of suspended particulate matter (SPM) on a deep station in the Laurentian Trough, Gulf of St Lawrence (48° 42.2' N, 68° 39.5' W). *a*, Manganese in May; *b*, manganese in September; *c*, SPM in May; *d*, SPM in September.



**Fig. 2** Vertical distribution of manganese in a sediment core from the Laurentian Trough, Gulf of St Lawrence (48° 40.8' N, 68° 38.8' W). The core was taken with a box corer, subsampled immediately, and analysed by the method of Rantala and Loring<sup>17</sup>.

way. As the exported manganese is predominantly in particulate form, it seems that the exported particulate matter is enriched in manganese. Furthermore, the existence of individual particles, greatly enriched in manganese, has recently been demonstrated in suspended matter from the Atlantic Ocean<sup>8</sup>. Using scanning electron microscopy and an electron probe, it was shown that the particulate matter is composed of several phases of different manganese concentrations, so that the bulk composition is controlled by the relative abundance of these phases. Thus, manganese was enriched to about 10 times the normal level in clay particles that make up 10% of the total clay.

If production and export of manganese-rich particulate matter occurs generally in the coastal zone, it will have interesting implications for oceanic geochemistry. Manganese and other trace elements are not homogeneously distributed in deep-sea sediments. Thus, the highest concentrations are not found in clays from the abyssal plains adjacent to the continents, but from elevated areas remote from the continents. To explain this, Turekian<sup>9</sup> distinguished between two sediment transport mechanisms: bottom transport and deposition, and eupelagic deposition. The continentally remote hilltops contain material derived from the higher parts of the water column, that is the eupelagic components, and the abyssal plains adjacent to the continents contain the bottom transported components in addition. If very fine particles, rich in the critical trace elements, were transported by the surface layers of the oceans, then a uniform 'rain' of trace elements in particulate form would occur over the entire ocean. The bottom transported components would act as a diluent, causing the lower levels of trace elements observed in sediments adjacent to the continents.

The major problem with the theory is the origin of manganese-rich particles. Turekian<sup>9</sup> suggested that the fine particles could either be manganese oxide produced during the weathering of rocks, or small particles with high specific surface area which had absorbed the critical trace elements during stream transport. The difficulty with this is the implication that the removal of trace elements such as Ni, Co and Mn from seawater is of secondary importance to their removal in the river environment. The removal

of trace elements from seawater, and the maintenance of steady-state conditions, is thought to occur through scavenging by particulate matter settling through the water column<sup>10,11</sup>, and in particular by hydrous oxides of manganese in view of their high capacity for absorbing metal ions<sup>12</sup>. As Turekian<sup>9</sup> has pointed out, it is doubtful if particles transported by streams, be they manganese oxide or clay particles, are efficient scavengers of trace metals from seawater. As the concentrations of most trace elements are as high in streams as in seawater, trace elements would most likely have been absorbed to capacity on the stream particles. The higher sodium content of seawater would tend to inhibit further adsorption of trace elements from seawater, if not actually cause their release.

A marine, rather than river, origin of manganese-rich particles would remove this difficulty. These particles, with their content of freshly precipitated manganese oxide, would be the ideal scavenger, helping maintain the low concentrations of dissolved trace metals in seawater.

Another implication comes from massbalance calculations which involve manganese. Elderfield<sup>13</sup>, in a recent attempt to draw up a budget for oceanic manganese, concluded that the rate at which rivers supply dissolved manganese to the ocean is about an order of magnitude less than the rate at which non-lithogenous manganese accumulates in pelagic sediments. Hence, other significant sources of non-lithogenous manganese must exist.

One such source, if liberated by near-shore diagenetic processes, could be the non-lithogenous fraction in river-borne detritus, that is absorbed and complexed manganese and manganese oxide coatings. The riverborne detritus, of which some 95% never reaches the open ocean<sup>14</sup>, could then be considered a reservoir for manganese, available for remobilisation. The annual input of riverborne detritus to the marine environment is about  $2 \times 10^{16}$  g (ref. 15). If, from the total manganese carried by the detritus (estimated at an average of 4,300 p.p.m. (ref. 16)) as little as 50 p.p.m. were on average remobilised, that alone would amount to a flux of  $10^{12}$  g yr<sup>-1</sup>. This is an order of magnitude higher than the dissolved manganese supplied by the rivers, indicating the potential significance of this source.

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1. Goldberg, E. D. & Arrhenius, G. *Geochim. cosmochim. Acta* **13**, 153–212 (1958).
2. Rantala, R. R. & Loring, D. H. *At. Absorp. Newslett.* **16**, 51–52 (1977).
3. Sundby, B. *Can. J. Earth Sci.* **11**, 1517–1533 (1974).
4. Feely, R. A. *Mar. Chem.* **3**, 121–156 (1975).
5. Evans, D. W., Cutshall, N. H., Cross, F. A. & Wolfe, D. A. *Estuar. Coast. Mar. Sci.* **5**, 71–80 (1977).
6. Graham, W. F., Bender, M. L. & Klinkhammer, G. P. *Limnol. Oceanogr.* **21**, 665–673 (1976).
7. Bewers, J. M. & Yeats, P. A. *Nature* **268**, 595–598 (1977).
8. Chesselet, R., Spencer, D. W. & Biscaye, P. E. *Special Symp. Geochemistry and Ocean Mixing. Joint Oceanographic Assembly* (Edinburgh, 1976).
9. Turekian, K. K. in *Progress in Oceanography* (ed. Sears, M.) 227–244, (Pergamon, Oxford, 1967).
10. Goldberg, E. D. *J. Geol.* **62**, 249–265 (1954).
11. Turekian, K. K. *Geochim. cosmochim. Acta* **41**, 1139–1144 (1977).
12. Jenne, E. A. *Am. Chem. Soc., Adv. Chem. Ser.* **73**, 337–387 (1968).
13. Elderfield, H. *Mar. Chem.* **4**, 103–132 (1976).
14. Broecker, W. S. *Chemical Oceanography* 97–103 (Harcourt, Brace Jovanovich, New York, 1974).
15. Goldberg, E. D. *Comments Earth Sci. Geophys.* **1**, 117 (1971).
16. Aston, S. R. & Chester, R. in *Estuarine Chemistry* (eds Burton, J. D. & Liss, P. S.) 37–52 (Academic, London, 1976).
17. Rantala, R. R. & Loring, D. H. *At. Absorp. Newslett.* **14**, 117–119 (1975).

## Equilibrium concentrations of methylmercury in Ottawa River sediments

MOST of the mercury (up to 97%) found in aquatic systems is associated with bed sediments<sup>1</sup>. Fish, invertebrates and plants contain only 0.02% of the total, though they contain a high proportion of organic mercury. Field surveys show that the proportion of organic mercury in the sediments range from

0.1 to 2.1%<sup>2–6</sup>. Jacobs and Keeney placed river sediments mixed with inorganic mercury in the Wisconsin and Fox Rivers<sup>7</sup>. After 12 weeks exposure to the natural environment the concentration of methylmercury in the sediments was only 3%. This concentration of methylmercury was attained within 4 weeks after sediments were placed in the bottom of the rivers. This indicated that the processes of methylation and demethylation of mercury were in equilibrium in these environmental conditions within 4 weeks. But no experiments were conducted to show the degradation of methylmercury in sediments. After the methylation study by Jensen and Jernelöv<sup>8</sup>, many investigations into methylmercury production by micro-organisms in bed sediments have been carried out. Little attention, however, has been paid to the ratio of methylmercury to the total mercury existing in the sediments. Spangler *et al.* observed degradation of methylmercury in mixed cultures from sediments<sup>9</sup>. The degradation reached 50% within 5 d, they also found methylmercury degradation in sediments. But detailed information about the sediments was not available and results fluctuated wildly. We do not know the total amount of methylmercury existing in all components of the systems, including fish, invertebrates and plants as well as various types of bed sediments. Here we use methylmercury equilibrium concentration levels in the Ottawa River sediments to estimate the amount of methylmercury in various types of bed sediment where detailed information of total mercury concentrations (total mercury estimation 31 kg) and types of sediment is available. Two ways (methylmercury production and degradation) of reaching equilibrium were observed during 50 d in identical environmental conditions.

Three types of fresh sediments from the study section of the Ottawa River were spiked with either radioactive mercuric chloride or methylmercuric chloride at a level of 1.00 p.p.m. The three sediments were: (A) main stream sediments, (B) north channel sediments, and (C) wood-chip sediments which were located in the north channel but just down-stream of the pulp and paper company. Original total mercury concentrations in the three types of sediments were determined by atomic absorption spectrometry as 17, 23 and 1,600 p.p.b., for type A, B and C sediment, respectively. Therefore, final concentrations of total mercury in type A, B and C sediments were 1,017, 1,023 and 2,600 p.p.b. More detailed information about these sediments is given elsewhere<sup>10</sup>.

Because no isotopic exchanges were observed between organic and inorganic mercury under natural environmental conditions and in these mercury concentration ranges<sup>11</sup>, it was possible to consider that increase or decrease of radioactive methylmercury in sediments was caused by methylation or demethylation not by simple isotopic exchanges. Organic contents of these sediments (A, B and C) were 0.35, 0.84 and 59.2%, respectively. The sediments were placed in the dark at 20° C and closed to prevent a loss of moisture as well as of mercury from the sediments.

Thin layer chromatography (TLC) and atomic absorption were used to determine the quantities of the various chemical forms of mercury, mainly mercuric and methylmercuric as well as the total mercury in sediments. A modification was made to extract various forms of mercury from sediments using 0.1% dithizone–benzene solution. Over 98% of the mercury from sediments was recovered using this modified method. *R<sub>f</sub>* values in the thin layer chromatography for mercuric and methylmercuric forms, respectively, were 0.36 and 0.66 using water–methylcellulose as the developing solvent. A portion of the mercuric and methylmercuric bands as well as other bands containing mercury were separated from the TLC sheet and were counted for radioactivity. All samples were analysed in duplicate.

Surprisingly, methylmercury in cleaner sediments (type A, main channel sediments (sands)) degraded more rapidly into inorganic forms, Fig. 1. Within 25 d, 80% of methylmercury was broken into inorganic forms in the sand sediments, whereas, only 14% of methylmercury was converted into

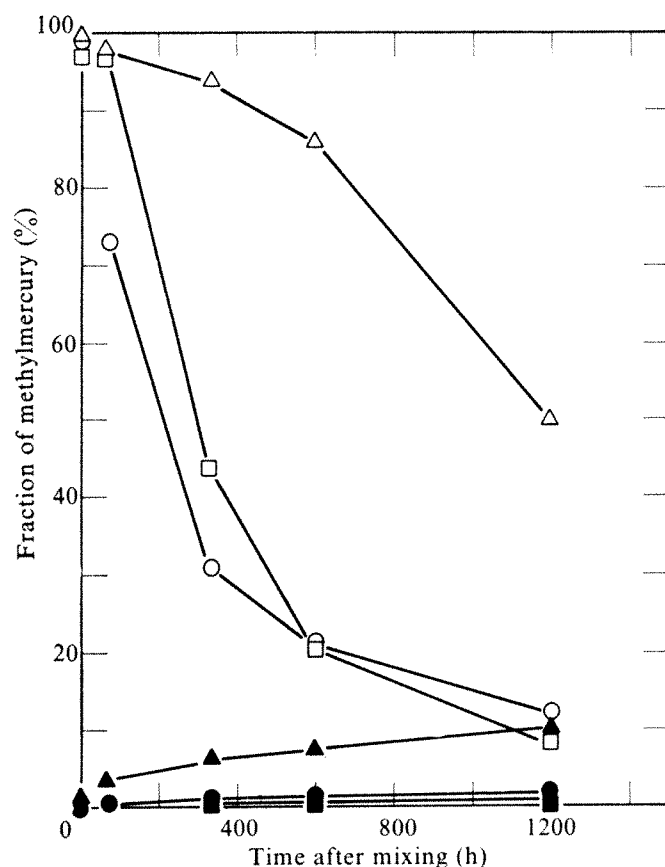


Fig. 1 Equilibrium concentration of methylmercury in Ottawa River sediments. Sediment A is shown by squares, B by circles and C by triangles, closed symbol, start from mercuric chloride only; open symbol, start from methylmercuric chloride only.

inorganic forms in the organic sediments (type C) during the same period. Sediments which were mixed with an inorganic form of mercury increased their contents of methylmercury with time. For highly organic contaminated sediments (type C, wood-chip sediments) the portion of methylmercury was 7.4% and for cleaner sand (type A, main channel sediments) the portion was only 0.1% after 25 d. The difference in final methylmercury concentration between sand (A) and wood-chip (C) sediments is probably a result of the different rates of formation and decomposition of methylmercury in these sediments. That is, the wood-chip sediment forms methylmercury more rapidly and also decomposes methylmercury more slowly than does the sand sediment. During the 50-d experiments, an average of 1.3% of mercury escaped from the system.

Methylmercury concentrations in each of the three types of sediments converged to an equilibrium concentration level from both directions starting with mercuric chloride and methylmercuric chloride, with an increase of time, Fig. 1. From the results, the equilibrium concentration levels were estimated for sediments A, B and C as 0.2, 2.3 and 12.1%, and the time to attain the equilibrium concentration was estimated as 3, 3, and 6 months, respectively.

Applying this methylmercury information to Ottawa River sediments, amounts of methylmercury were calculated as 1,200 g for the study section of the Ottawa River in 1972. In this calculation, the 4.88 km by 1.5 km section was divided into five regions depending on the type of bed sediments existing in the section of the river. The amounts of biomass in the study section were 5.3, 11.1, and 65.4 tons (wet) for fish, invertebrates, and plants, respectively. This biomass contained only 6.0 g of total mercury. In other words, bed sediments contained 200 times more methylmercury than those in biomass, even assuming all of the mercury in biomass was

methylmercury. Of course, the accuracy of this calculation of methylmercury in the sediments was not as high as that for the estimation of the total mercury in biomass in the study section. The calculation clearly showed, however, that methylmercury existing in the sediments was higher by at least two orders of magnitude, than those in the biomass. This further indicates that a detailed study must be directed toward mercury in bed sediments to understand and control, effectively, mercury pollution in aquatic systems.

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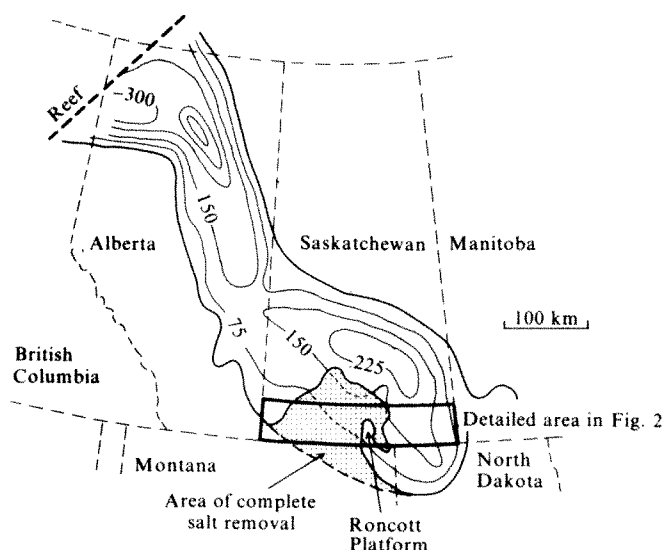
1. Kudo, A., Townsend, D. R. & Miller, D. R. *Prog. wat. Tech.* 9, 923 (1977).
2. Andren, A. W. & Harris, R. C. *Nature* 245, 256 (1973).
3. Batti, R., Magnaval, R. & Lanzola, E. *Chemosphere* 4, 13 (1975).
4. Langbottom, J., Pressman, R. & Lichtenburg, J. J. *Ass. Off. analyt. Chem.* 56, 1297 (1973).
5. Olson, B. H. & Cooper, R. C. *Nature* 252, 682 (1974).
6. Eganhouse, R. P. *Southern California Coastal Water Research Proj. Rep.* 83 (El Segundo, California, 1976).
7. Jacobs, J. W. & Keeney, D. R. *J. environ. Quality* 3, 121 (1974).
8. Jensen, S. & Jernelöv, A. *Nature* 223, 753 (1969).
9. Spangler, W. J., Spigarelli, J. L., Rose, J. M. & Miller, H. M. *Science* 180, 192 (1973).
10. Kudo, A., Townsend, D. R. & Miller, D. R. *J. Am. Soc. Civil Engng. Env. Div.* 103, EE4, 605 (1977).
11. Kudo, A., Akagi, H., Mortimer, D. C. & Miller, D. R. *Environ. Sci. Technol.* 11, 907 (1977).

## Origin of coal basins by salt solution

WILLISTON Basin of North America, one of the world's largest brown coal basins with diameter 550 km, is interpreted as an effect of regional solution of up to 150 m thickness of Devonian salt. The Late Cretaceous-Tertiary salt removal from the buried Elk Point Evaporite Basin (Middle Devonian) has resulted in sufficient collapse subsidence to be structurally reflected in the 2,000–3,000 m of overlying Palaeozoic and Mesozoic stratigraphic column. The Palaeocene coalfields are topset to Late Cretaceous delta lobes developed within salt solution troughs. This syndepositional mechanism for coal basin formation has not previously been recognised.

The most extensive coal basins were emplaced marginal to the Roncott Platform<sup>1-3</sup>, a major salt salient into the 2,500 km<sup>2</sup> of complete salt removal across southern Saskatchewan (Figs 1 and 2). Ground water circulation believed to have caused the salt solution is attributed to flow under hydraulic head from the nearby Rocky Mountains which were emerging to the southwest. Initially, it was directed along vertical planes associated with rejuvenated Precambrian lineaments, which are related to a possible plate boundary<sup>4</sup> that trends across western South Dakota and eastern Montana and traceable into south-central Saskatchewan as the salt solution axes flanking the Roncott Platform.

The Palaeocene coal measures are coal-bearing bodies that are topset to the Cretaceous delta lobes. Wood Mountain-Willow Bunch complex is the largest<sup>5</sup> of the coalfields (Fig. 2). Its several depositional troughs are along the western margin of the Roncott Platform (Figs 2 and 3). The thicker parts of seams overlie anomalous negative troughs on the Late Cretaceous structural surface, which themselves partially represent syndepositional clastic thickening trends. The coal seams of the Wood Mountain and Willow Bunch coal basins have their thicker parts superimposed. These are in turn vertically above the Late Cretaceous delta lobes' axial lengths. Without the underlying solution subsidence any thick peat accumulation associated with temporary mobile axes would not be preserved



**Fig. 1** Isopachous map of the Prairie Evaporite Formation (Devonian) salt and potash beds in the Elk Point Evaporite Basin across western Canada. Contour interval is 75 m. The salt beds to the south-east were leached, resulting in evaporite-solution brecciation of superjacent strata, especially during the Late Cretaceous to Lower Tertiary. The resulting extensive collapse subsidence over this zone was reflected up the 2,000–3,000 m stratigraphic column during the emplacement of Williston Basin's deltaic topset coal basins, whose preservation was assured within the subsidence troughs. The best developed coal basins were emplaced marginal to the Roncott Platform, a salt salient that extends northwards from northeastern Montana into the leached zone (Fig. 2).

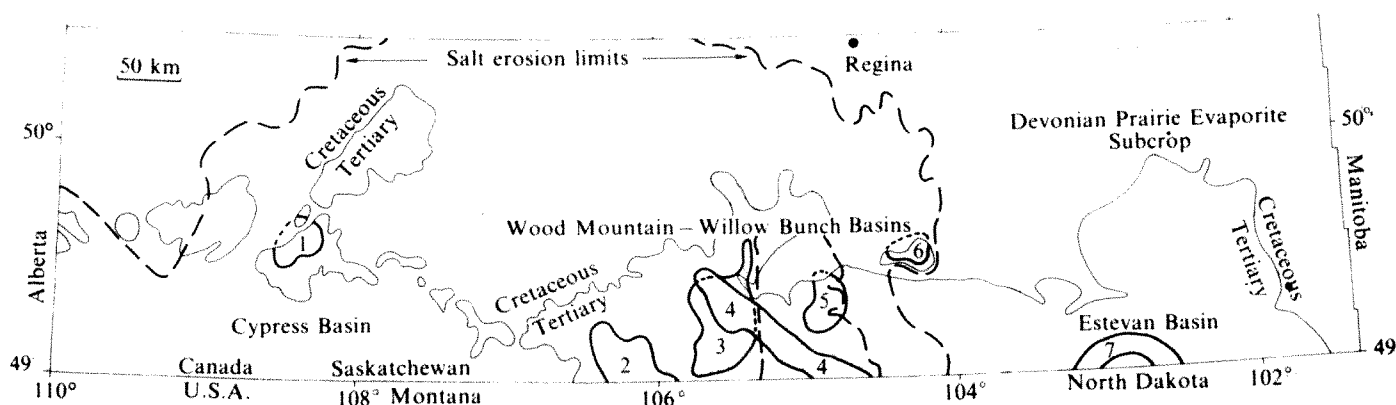
in the record. This phenomenon of coal basin genesis is well illustrated by deposition within the Coronach Trough, marginal to the western flank of the Roncott Platform (Fig. 2). There are six major seam groups in the Ravenscrag Formation of the Coronach Trough. The thickest seam of the 80–120 m coal measures in the trough, as well as in the formation, is the Hart seam. Its thickened trend, the 3–10 m thickness isopach interval, is a north-south subcrop belt, 8–24 km wide, that parallels the 65 km length of the Roncott Platform salt subcrop erosional edge north of the International Boundary (basin 3, Fig. 2). The Hart seam thins eastward in the subcrop along a broad front paralleling this salt erosional edge such that the

thinned depositional margin of the seam basin and the Roncott Platform western erosional edge coincide in the vertical succession along most of its length. Where the seam encroaches on to the salt platform subcrop zone, it is less than 1-m thick. All seams in the coal measures that are of sufficient thickness to be correlative between boreholes in the subcrop have their axes of maximum thickness superimposed, paralleling the Roncott Platform erosional margin and vertically along the maximum thickness trend of the Hart seam. They similarly thin eastward along a broad front as they encroach the salt substrate. The uppermost seam group of the Willow Bunch Basin, the 4–7 m thick seams of the Willow Bunch group (basin 4, Fig. 2) are exceptional in that they transect the Roncott Platform; their deposition axes are oblique to the Coronach Trough subsidence. The isopachous trends of Willow Bunch seams demonstrate the markedly diminished influence of salt solution subsidence emplacement and the assertion of the regional cratonic southeastern subsidence in the syntectonic sedimentological framework.

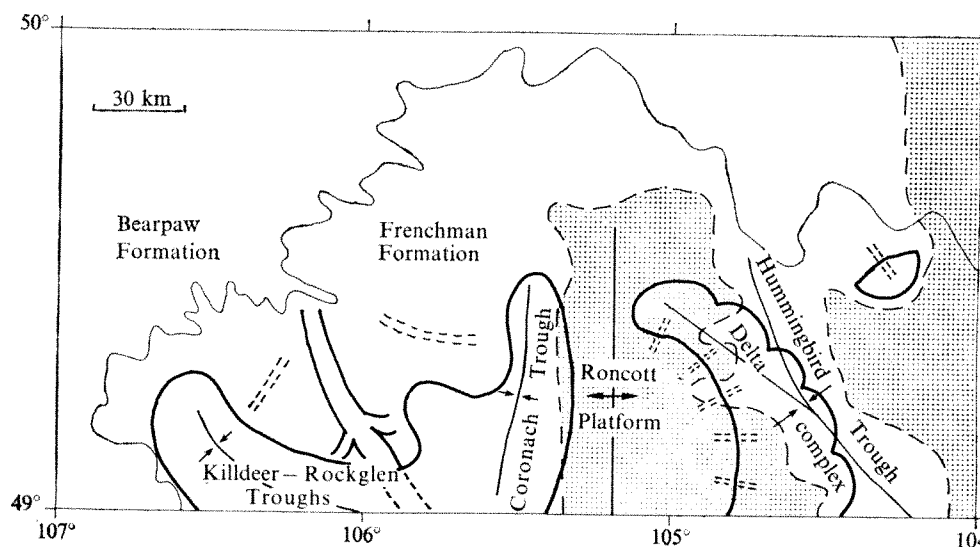
The salt solution subsidences of the Killdeer–Rockglen and Coronach Troughs (Fig. 3) were contemporaneous with the final recession of the Bearpaw sea. They favoured the fixation of vertically accreting Late Cretaceous deltaic lobes within a Catskill-like<sup>6</sup> muddy shoreline that transgressed over the sea. The main delta body, approximately 100 km across, extends south of the International Boundary beyond the study area (Fig. 3), and is comparable in size to the largest of deltas observed in the sedimentary record world-wide. The delta lobe accretion along subsidence axes inhibited lateral progradation and developed the Palaeocene coal measures as topset beds: the margins of the largest seam basins coincide with the subcrop extent of the late Cretaceous lobe. This sequence is repeated with modification eastward into the Hummingbird Trough along the eastern margin of the Roncott Platform (Fig. 3). Sufficient but incomplete removal of evaporite beds contemporaneous with the Late Cretaceous continental transgression across the eastern Platform developed a series of coalesced 5–10-km diameter lobes (Fig. 3) that encroached the western limb of the Hummingbird Trough. The relatively small Bengough coalfield (basin 5, Fig. 2) in the overlying Palaeocene thus is situated on this semi-stable Roncott salt platform area, but its thick seams do not laterally extend into the axial area of the Hummingbird Trough. This may be due to unfavourable subsidence for accumulation and preservation of peat sections.

The few major intracratonic deltas studied in the ancient sedimentary record, for example, the Upper Cretaceous delta of

**Fig. 2** The late Tertiary and Pleistocene drift blankets the unconformably Late Cretaceous to Early Tertiary bedrock surface on the northern limb of the Williston Basin, southern Saskatchewan. Most of the Palaeocene coal basins of this borehole exploration area are emplaced within salt solution subsidence zones: Cypress (1) Wood Mountain (2) and portions of the Willow Bunch (3–6) Basins. The lower coal measures of the Coronach Trough (3) are emplaced to offset to the paralleling western margin of the Roncott Platform's rigid salt substrate, whereas the Bengough coalfield (5) is developed with the partial solution subsidence of the eastern half of the salt structure. Minor coal basins (6) are emplaced within embayments into the salt substrate along the eastern Hummingbird Trough (Fig. 3) contact with the regional salt substrate. The stratigraphically high Willow Bunch seam group basin (4) is the least affected by the solution subsidence, and transects the subjacent solution axis. Likewise, the equivalently young Estevan Basin (7) coal measures develop with the assertion of cratonic subsidence as the dominate syndepositional genesis of coal basins in place of solution subsidence.







**Fig. 3** Emplacement of the Late Cretaceous delta lobes along salt solution subsidence axes within the Frenchman formation. These lobes persisted in the vertical succession as topset Palaeocene coal basins. Extensive progradation of the main lobe along the Coronach axis was inhibited by the structural barrier of rigid salt substrate (stippled) of the western Roncott margin.

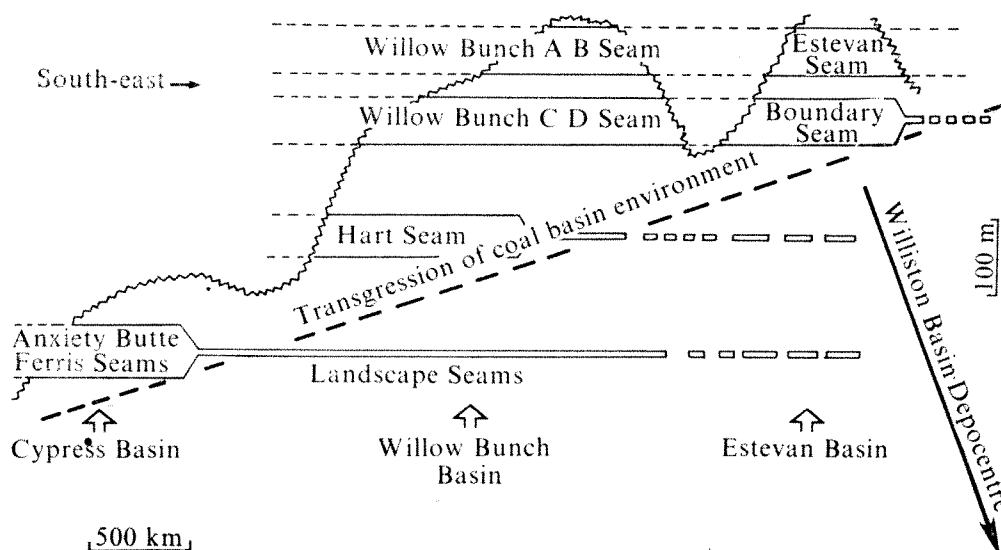
the central Rocky Mountains<sup>7-8</sup>, have been created by crustal downwarping reinforced by sediment loading. By contrast, solution subsidence as the cause of the Late Cretaceous to Lower Tertiary Wood Mountain-Willow Bunch delta complex suggests that loading is of minor importance. The crustal movement along the postulated geosuture was primarily lateral with minimal vertical component. Such movement does not preclude the development of vertical fracture zones into the Phanerozoic, including the Devonian salt beds to promote channelisation of sufficient fluids for regional dissolution.

The initiation of thick seams in the coal measures on the northern limb of the Williston Basin is transgressive southeastward towards the depocentre in northwestern North Dakota (Fig. 4). The thick seams of the Cypress Basin are at the base of the Tertiary (Ravenscrag Formation); the Ferris and superjacent Anxiety Butte seams, each developed to between 4 and 8 m thick. This interval is approximately isochronous with the relatively thinned Landscape seams, tens of metres higher in the stratigraphic section of the Coronach Trough, 200 km to the east. The Willow Bunch Basin subsidence did not diminish, to a rate permitting thick peat accumulation until the onset of the Hart seam 100 m higher in the section (Fig. 4). Similarly,

these conditions were not developed in the Estevan Basin, another 200 km to the east, until an additional 100 m of section was deposited above thinned and discontinuous equivalents to the Hart seam. The Boundary seam, the lowest of the thick seams in the Estevan Basin coal measures is isochronous on the basis of palynology with the main thick seam of the stratigraphically high Willow Bunch group across the Willow Bunch Basin (Fig. 4).

The onset of subsidence rates favourable for accumulation and preservation of thick peat sections is interpreted as a linear transgression up section as distal limb sections detach from the relatively rapid but diminishing subsidence of the cratonic basin. This is contemporaneous with the transition up section from subsidence dominated by salt solution to sufficiently diminished cratonic subsidence favourable for accumulation of thick peat sections in stagnant basins.

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**Fig. 4** Representation of the up section accumulation of the thick coal seams as a linear transgression of detached distal limb sections towards the craton depocentre.

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1. Holter, M. *Sask. Dep. Min. Res. Rep.* **123**, 113 (1969).
2. Gorrel, H. & Alderman, G. *Geol. Soc. Am. Spec. Paper* **88**, 291–317 (1968).
3. Smith, D. & Pullen, J. *Bull. Can. petrol. Geol.* **15**, 468–482 (1967).
4. Camfield, P. & Gough, D. *Can. J. Earth Sci.* **14**, 1229–1238 (1977).
5. Broughton, P., Irvine, J. & Whitaker, S. *Sask. Geol. Soc. Spec. Publ.* **2**, 81–94 (1975).
6. Walker, R. & Harms, J. *J. Geol.* **79**, 381–399 (1971).
7. Audley-Charles, M., Curran, J. & Evans, G. *Geology* **5**, 341–344 (1977).
8. Weimar, R. *Soc. Econ. Paleon. Mineral. Spec. Publ.* **15**, 270–292 (1970).

## Primitive lead in an Australian Zn–Pb–Ba deposit

RADIOACTIVE decay of  $^{238}\text{U}$ ,  $^{235}\text{U}$  and  $^{232}\text{Th}$  yields  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ , and  $^{208}\text{Pb}$ , respectively, as daughter products. This radiogenic lead is continually added to common lead in the source environments of ore deposits and the lead isotopic ratios of the resulting mixture are consequently time dependent. Because the relative proportions of these lead isotopes are fixed when the lead, common and radiogenic together, is separated from the parental uranium and thorium and incorporated into a lead deposit, determination of the isotope ratios provides evidence regarding the age of the lead ore and thus indirectly of the rock in which the ore is contained. Generally speaking, the older the ore the less radiogenic will be its lead. We report here analyses of extremely primitive lead from a small lead-bearing deposit in Western Australia.

The Big Stubby Zn–Pb–Ba deposits, located 5 km south of Marble Bar in the Pilbara Goldfield of Western Australia, have recently been described by Reynolds *et al.*<sup>4</sup> and their similarities to the Kuroko deposits have been discussed by Brook<sup>1</sup>. The conformable sulphide–sulphate deposits occur in an Archean volcanic sequence consisting of rhyolite breccias, rhyolite, and intermediate volcanics. Sphalerite and galena, dispersed within a barite gangue, are associated with a chert layer overlying a number of small rhyolite domes. The entire volcanic belt is intruded by granitic rocks dated at  $3.125 \pm 366$  Myr (ref. 3).

Five individual sulphide lenses are known at Big Stubby but only one, the 'A' Lens, has been systematically drilled where the best intersection is 4.27 m of 0.34% Cu, 4.5% Pb, 25.0% Zn, 1.071 g Ag per tonne and 15% Ba. Four intersections have been made in the 'A' Lens over a length of 500 m and the average grade is 0.26% Cu, 4.6% Pb, 16.5% Zn, 533 g Ag per tonne and 17% Ba.

It should be noted that the largest known barite deposits in Western Australia occur only 40 km WNW of Big Stubby and

**Table 2** Average isotopic compositions of lead from Old Star, Letaba and Big Stubby

Deposit	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	Ref.
Old Star	12.214	13.851	32.16	5
Letaba	12.679	14.089	32.51	5
Big Stubby	11.95	13.72	31.94	This work

have also been described as conformable deposits associated with sedimentary chert layers in an Archean volcanic sequence<sup>2</sup>.

Lead isotope analysis of three galena samples from Big Stubby are shown in Table 1. A significant feature of the Big Stubby lead is that it is the least radiogenic 'ore lead' reported to date, although feldspar from the Amitsoq gneiss of West Greenland contains lead which is even less radiogenic<sup>7</sup>. It is considerably more primitive than lead in galena from carbonate veins in the Old Star gold mine, South Africa, previously considered to contain the least radiogenic lead<sup>5</sup>. The same authors report that lead from Letaba, a subeconomic strata-bound massive pyritic deposit, although more radiogenic than that in the Old Star vein, is the least radiogenic found in a conformable deposit. It now seems that Big Stubby contains lead more primitive than either of these. Furthermore, an average of the Big Stubby data yields a model lead age of about 3,500 Myr according to the Stacey–Kramers model<sup>6</sup>.

For comparison, average isotopic compositions of lead from Old Star, Letaba and Big Stubby are presented in Table 2. Further lead and sulphur isotopic analyses of samples from this ancient segment of the earth's lithosphere are underway.

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1. Brook, W. A. 45th *Anzacs Congress*, Perth Western Australia, August 13–17, 1973. Section 3, *Geology*, Symposium 8, Paper 8.8 (Abstr.) (1973).
2. Hickman, A. H. in *Geological Survey Western Australia, Annual Report 1972* 57–60 (1973).
3. de Laeter, J. R. & Blockley, J. G. *J. geol. Soc. Aust.* **19**, 363 (1972).
4. Reynolds, D. G., Brook, W. A., Marshall, A. E. & Allchurch, P. D. *Aust. Inst. Min. Met. Mon. Series No. 57* 185–195 (1975).
5. Saager, R. & Koppel, V. *Econ. Geol.* **71**, 44–57 (1976).
6. Stacey, J. S. & Kramers, J. D. *Earth planet. Sci. Lett.* **26**, 44–57 (1975).
7. Baadsgaard, H., St. J. Lambert, R. & Krupicka, J. *Geochim. cosmochim. Acta* **40**, 513–527 (1976).
8. Stacey, J. S., Delevaux, M. H. & Ulrych, T. S. *Earth planet. Sci. Lett.* **6**, 15–25 (1969).

## East Anatolian earthquake of 24 November 1976

A DESTRUCTIVE earthquake occurred north of Van in East Anatolia on 24 November 1976 at 12:22:18.3 GMT (USGS epicentre: 39.10°N and 44.02°E; average magnitude:  $M_s = 7.3$ ). The earthquake did extensive damage, destroyed more than 80% of the dwellings in a 2,000 km<sup>2</sup> area and caused more than 4,000 deaths. In this area of complicated geology<sup>1–3</sup>, this was the only known large earthquake in the last century according to seismicity catalogues<sup>4–5</sup> and the recollection of villagers. The fault trace and the displacement were clearly visible and could be mapped for the total length of the fault. Furthermore, any precursory phenomena that might have preceded the earthquake as perceived by the local residents along the whole fault could be investigated. We summarise here the results of extensive field investigations carried out during the first 10 days immediately following the earthquake, and again in July 1977.

The location of the earthquake relative to major faults and earthquake epicentres is shown in Fig. 1. The earthquake is to the

**Table 1** Lead isotope analysis of galena samples from Big Stubby

Sample no.	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$
Sp-3523	$11.95 \pm 0.09$	$13.74 \pm 0.10$	$31.96 \pm 0.24$
SP-3525	$11.97 \pm 0.09$	$13.74 \pm 0.10$	$31.93 \pm 0.24$
SP-3527	$11.93 \pm 0.09$	$13.69 \pm 0.10$	$31.92 \pm 0.24$

Analyses were performed by Teledyne Isotopes, Inc., under the direction of L. Casabona. The data are corrected to absolute values on the basis of measured and absolute values for standard lead SRM 981<sup>8</sup>. The approximate 95% confidence level, based on the analysis of 20 duplicate samples, is 0.76%.

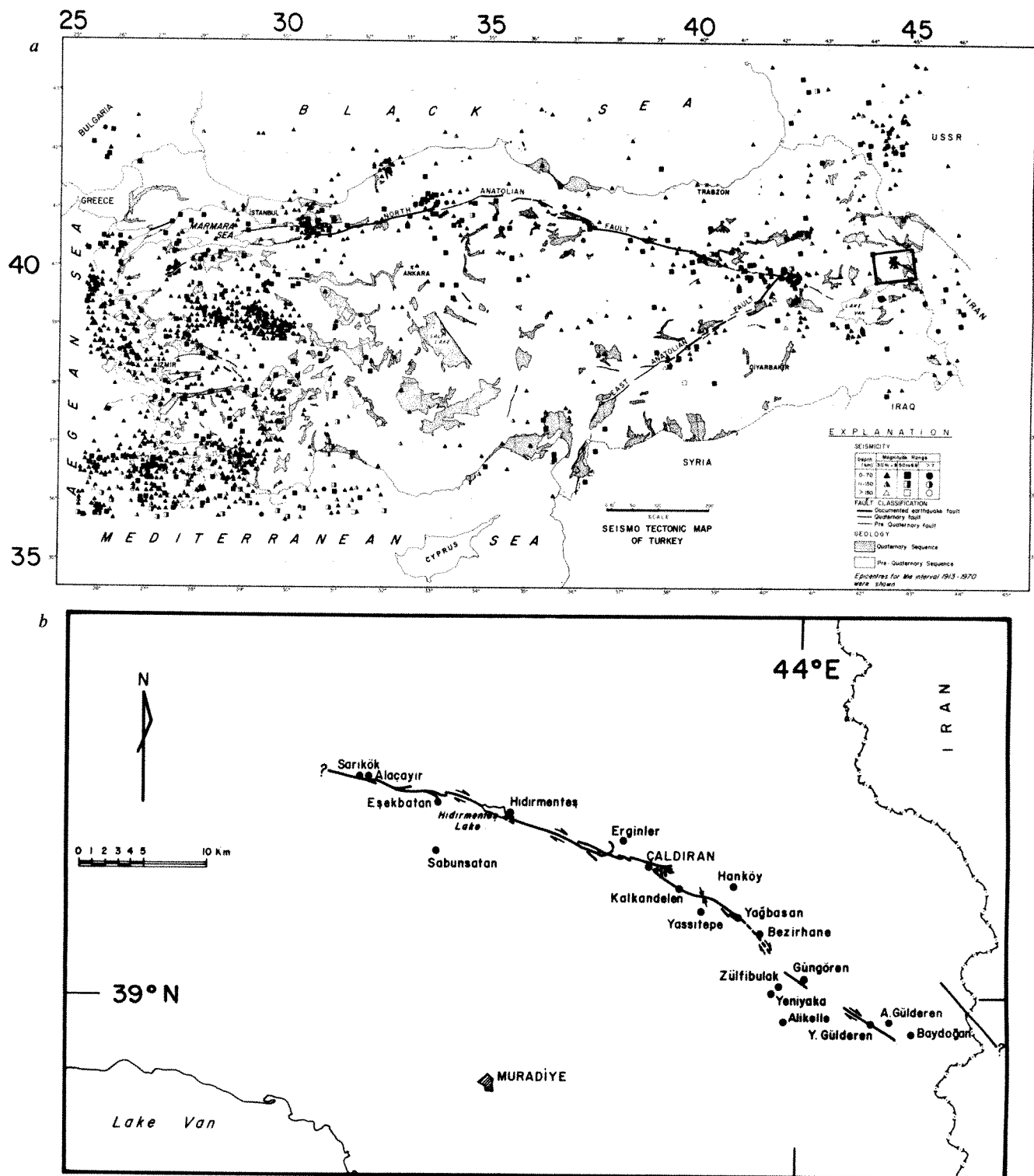


Fig. 1 *a*, Location of the earthquake within the seismotectonic setting of Turkey (inside the box), and *b*, detailed map of the observed fault trace.

east of the junction of the North Anatolian and East Anatolian Faults. The observed fault trace is to the north of a possible southeastern extension of the N. Anatolian fault trace through Lake Van. The connection of this earthquake fault with those in Iran is not clear, although there are a number of right-handed, strike-slip faults in Iran (branches of the North Tabriz fault) extending toward the Turkish border<sup>6</sup>. There are young volcanoes in the area and Tendurek, only 20 km north of the epicentre, has been active in the quaternary period. A more detailed description of the geology and tectonics of the region is given by Arpat *et al.*<sup>7</sup>.

The fault trace was clearly visible for about 50 km (Figs 1*b*, 2). The motion was almost purely right-lateral, strike-slip. The surface trace has a strike of about N 70°W in the central and western side and it bends somewhat east of Caldiran and continues along an azimuth of about 135°. The surface break is more prominent in the central and western sections, and has some *en-echelon* jumps in the east. The fault disappears abruptly under mountains in the west. At the eastern end the disappearance is gradual. The fault trace splinters and disappears toward the Iranian border. The observed horizontal displacements are more than 3 m (330–350 cm) in the west, about 250 cm in the central



regions and less to the east. The dip is nearly  $90^\circ$ . There are vertical displacements with inconsistent direction, generally about 50 cm, observed locally in several areas. The fault plane solution gives a right-handed, strike-slip source mechanism. The photographs in Fig. 2 show the fault trace and clearly document the right-handed motion. From the distribution of displacements, it seems that faulting may have started close to the west end and propagated to the east. The reported duration of severe shaking in Van was about 17 s, consistent with a fault length of about 50–60 km.

Seismic moment and stress drop can be calculated from the field observations. Taking the fault length  $L = 55$  km, assuming fault width  $W = 1/3 L$  and the average observed displacement  $D = 250$  cm, and rigidity  $\mu = 4 \times 10^{11}$  dyn cm $^{-2}$ , the seismic moment is  $\bar{M} = 1.0 \times 10^{27}$  dyn cm. The stress drop calculated using a strike-slip model is  $\Delta\sigma = 35$  bar. Comparing this value with those of other earthquakes, we find that the stress drop falls within the general range of those inter-plate earthquakes<sup>8</sup>. The observed displacements and calculated stress drop, however, are high as compared with some other strike-slip earthquakes. The relatively young age of the fault and the recent basalt flows across which the fault cuts may be responsible for this deviation.

There were no instruments in the area except for a seismoscope in Van, about 90 km to the south-west of the epicentre. A county centre, however, (Caldiran) and about 20 villages were located directly on the fault trace. The reports from these and other affected villagers were gathered (mostly through personal interviews) regarding the pre- and post-earthquake phenomena. The earthquake occurred at 2:22 p.m. local time. Most villagers raise livestock. About half the people were outdoors tending their herds. Because of high regional elevations, the weather was already cold and in the western end, the ground was covered with snow.

There were no foreshocks felt along the fault zone before the main shock on 24 November 1976. In two villages along the western half of the fault trace, where displacements were highest, noises resembling thunder were heard several times during the week preceding the quake. These could have been due to small foreshocks, even though the shaking was not felt. In the village of Hidirmen, directly on the fault trace and where it cuts the lake, there were reports of noises coming from the lake (foreshocks, gas release, microfracturing?) during the same period. There was no observable change in water level before the earthquake.

There was one reported case of change in ground water activity before the earthquake: in the village of Yukarigölder, near the eastern end of the fault trace, discharge from a spring increased. Also associated with this increase was seepage of oil that covered the water surface. There are cases of oil seepage in this general area.

After the earthquake, major changes in ground water conditions were noticed both near the fault trace and on the southern side. Some springs became muddy and stayed so for days. A few springs dried on the north side of the fault and a well went dry in the south. At Tuzla, 50 km south of the fault trace, discharge from the salt spring increased by a factor of 20 immediately after the earthquake and it was three times normal a week later. The north shore of Lake Van was uplifted relative to the south shore by about 16 cm. The southward tilting of the lake was most likely co-seismic, but a deformation before the earthquake cannot be ruled out.

Behaviour of domestic and farm animals before the earthquake was investigated extensively by interviewing many villagers and shepherds. In this primarily ranching area, there are cattle, sheep, goats, horses and dogs. The animals are housed in barns adjacent to the houses, although at the time of the earthquake most were outdoors. We inquired in widely scattered villages along the fault zone. There were no confirmed observations of unusual behaviour of farm animals, outdoors or indoors, before the earthquake. There was one report of animal restlessness in a village on the western end of the fault that reportedly lasted for months before and after the quake. This was not observed at neighbouring villages. The barking and howling



**Fig. 2** Photographs of the fault trace observed immediately after the earthquake. *a*, The fault trace photographed from a distance. *b*, The right-handed displacement is clearly visible by the 250-cm offset of a ditch.

dogs were widely reported in a number of villages located on the west, central and eastern parts of the fault. The reported timing, however, of these events varied from a few minutes to a few hours before the earthquake.

Since there had been no major earthquakes in this region for a few generations, the villagers in general had no ideas or preconceptions about earthquakes. We believe that the reports we received were unbiased. There was no wide-scale abnormal behaviour of farm animals before the earthquake, except possibly for the behaviour of dogs. Physical precursors were definitely observed before the earthquake.

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1. Ketin, I. *Bull. Min. Res. Explor. Inst. Turkey*, Ankara **66**, 23–34 (1966).
2. Arpat, E. & Şaroğlu, F. *Bull. Min. Res. Explor. Inst. Turkey*, Ankara **78**, 33 (1972).
3. McKenzie, D. *Geophys. J. R. astr. Soc.* **30**, 109–185 (1972).
4. Ergin, K., Güçlü, U. & Uz, Z. *Technical Unit. Istanbul. Mining Eng. Fac.*, Publ. No. 24, 74pp (1967).
5. Alsan, E., Tezucan, L. & Bath, M. *Tectonophysics* **31**, 13–20 (1976).
6. Berberian, M. *Geol. Survey of Iran, Report No. 39* (1976).
7. Arpat, E., Şaroğlu, F. & İz, H. B. *Yerüstü ve İnsan*, **2** (1) 29–41 (1977). In Turkish.
8. Kanamori, H. & Anderson, D. L. *Bull. seism. Soc. Am.* **65**, 1073–1095 (1975).



## Timing of continental growth and emergence

HARGRAVES<sup>1</sup> has proposed that the separation of the primordial shells of the earth into continents and oceans began only about 3,700 Myr ago and continued through most of Precambrian time, with the result that the bulk of the continents as we know them today did not emerge from the sea until 1,400–1,000 Myr ago. Here I review geological and geochemical data that indicate that the growth and emergence of the bulk of the present-day continents took place in or by  $2,500 \pm 200$  Myr ago.

There are many estimates of the thickness that the continents had reached by the late Archaean. First, studies of mineral assemblages and chemistry correlated with experimental data on 3,000 Myr high-grade rocks from Greenland, Scotland, Africa and India suggest they formed at depths of 30–50 km (refs 2–5) and, because the Moho is 35–40 km beneath them, the Archaean crust in these regions was probably at least of the order of 65–80 km thick<sup>3,8</sup> (this discounts the unattractive alternative of subsequent sialic underplating to make up the difference), comparable to the continental crust today in the Andes<sup>6</sup>. These calculations are not surprising if the major period of crustal growth took place in the late Archaean by Cordilleran plate margin activity<sup>5,7</sup>. Extensive thickening of the crust in the late Archaean could have been effected by a combination of thrust-nappe stacking and magma addition<sup>8–10</sup>. Second, for greenstone belts mostly in North America, Condie<sup>3</sup> calculated crustal thicknesses primarily in the range of 20–30 km from Rb–Sr data, and of 20 km from K<sub>2</sub>O–SiO<sub>2</sub> data. Potash and Rb–Sr indices of granitic rocks suggest crustal thicknesses equal to or more than 30 km at the time of granite emplacement late in the evolution of the greenstone belts. Consideration of the spacing between volcanic centres in the Abitibi belt in relation to the known dependence of modern volcano spacings on crustal thickness gives an Archaean crustal thickness of 35–45 km (ref. 11). In comparison with the thickness estimates from the high-grade regions these values are consistent with formation of greenstone belts in some primitive form of marginal basin environment<sup>12,13</sup>. In spite of various problems connected with the interpretation of geobarometry, the above estimates suggest that the major stage of crustal fractionation was completed by the end of the Archaean.

There are many reasons for regarding the Archaean–Proterozoic time boundary (some  $2,500 \pm 200$  Myr ago) as the most important turning point in the evolution of the continents<sup>14–16</sup>; it represents the changeover from a permobile regime to a platform-geosynclinal style of tectonics that begins to show strong similarities with that of modern times. Indications of the new-found stability of the early Proterozoic continents include the intrusion of transcontinental basic dyke swarms, and the first appearance of alkaline complexes, kimberlites, carbonatites and aulacogens that extend from continental margins into the interior of stable plates. But the best evidence of the widespread emergence of stable cratons in the early Proterozoic is the appearance of miogeoclinal and geosynclines with the first recognisable continental margin sequences.

Many Archaean cratons are bordered, or partly underlain, by early Proterozoic sedimentary basins—an indication that the cratons were positive emergent areas bordered by shorelines in the early Proterozoic. One of the best-documented studies of craton-basin relations is by Hunter<sup>17</sup>, who has followed the development of basins in the Kaapvaal craton of southern Africa from about 3,300 to 1,850 Myr ago. He showed that the rates

of vertical movement of the basins decreased with time, which is consistent with the observed increase in volume of finer clastics and non-clastics in successively younger basins, and also estimated the percentage increase in inundation of the Kaapvaal craton by shallow Proterozoic seas, which took place by episodic regressions and transgressions. The data indicate that the craton was an emergent area more in the Archaean than the Proterozoic, the peak of continental emergence being reached between 3,350 and 3,000 Myr ago. Exposed mountains were gradually eroded during the Proterozoic, increasing the availability of sialic clastic debris and enabling increasing transgressions by epicontinental seas. This picture is at variance with the proposals that the Archaean continents were submerged beneath a globe-encircling sea, that the height of mountains increased during the Proterozoic, and that continental emergence did not take place on a substantial scale until after 1,400 Myr ago in the late Proterozoic<sup>1</sup>.

**Table 1** Some early Proterozoic geosynclines and basins in the North American Shield

Succession	Abbreviation on Fig. 1	Thickness (km)	Ref.
Huronian Supergroup	H	~12.0	19
Animikie Group	A	7.5	19
Coronation Geosyncline	C	10.7	20
Circum-Ungava Geosyncline	CU	> 15.0	21
Ramah Group, Labrador	R	1.7	22
Baffin Geosyncline	B	?	23
Chibougamau Group	CB	1.1	24
Wollaston Lake Fold Belt	W	?	21
Thompson Fold Belt	T	?	21
Hurwitz Group (Kaminak Subprovince)	K	4.0	21
Medicine Bow Mountains, Wyoming	M	8.0	24

Hunter's data are echoed in a more general way by Watson's<sup>18</sup> review of vertical movements during the Proterozoic. She concludes that erosion of Archaean cratons down to the present level of exposure was completed in periods of no more than 300 or 400 Myr after their stabilisation, and that the cratons typically underwent very limited vertical movements after about 2,000 Myr ago.

The North American shield contains a large number of early Proterozoic geosynclines and basins (in the Aphebian age range, 2,560–1,800 Myr), some of which are shown in Table 1 (see also Fig. 1).

These Aphebian successions, many of which are unconformable, overlie or border Archaean cratons for a minimum total length of 8,000 km (Fig. 1). Besides iron formations and dolomites the sediments consist predominantly of clastic conglomerates, quartzites, siltstones, shales and sandstones. It is evident that over the whole shield Archaean mountains were being eroded to provide great thicknesses of terrigenous clastic debris in the early Proterozoic<sup>14,25</sup>. The Huronian is a miogeoclinal sequence containing craton-derived sediments on the south side of the Superior Province<sup>19</sup>. The Coronation Geosyncline has a stratigraphy and structure that so faithfully mirrors that of modern Cordilleran successions that it is highly likely that modern-style plate tectonics began by the early Proterozoic<sup>20</sup>. The Circum-Ungava Geosyncline probably formed in a narrow ocean or rift that was closed by continent–continent collision<sup>26,27</sup>. The North American successions are no doubt only remnants that were once more extensive, but, nevertheless, they are sufficient indication that the bulk of the Archaean shield was undergoing uplift and emergence in the early Proterozoic.

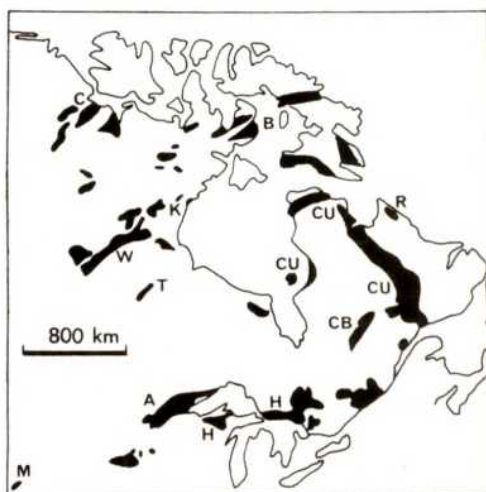


Fig. 1 Early Proterozoic geosynclines in the North American Shield. For abbreviations see Table 1. Compiled from refs 21, 24, 25.

There are well-known comparable early Proterozoic successions on all other continents, which abound in clastics and which indicate that Archaean cratons the world over were being highly eroded and many were at or near sea level by the early Proterozoic.

Rb-Sr and Pb-U isotope systematics yield information on the timing of fractionation of continental crust. For example, the growth of the strontium isotope ratio with time and the variable intercepts with the primary growth curve in the lead isotope system may be used to distinguish juvenile addition or accretion of mantle-derived material to the continental crust from rejuvenation or reworking of continental basement<sup>28,29</sup>. Table 2 shows estimates of the percentages of present-day continental crust formed by the late Archaean.

These values agree with estimates, based on areas of rock distribution, that at least 50% of North America<sup>34</sup> and Africa<sup>35</sup> were in existence by 2,500 Myr ago. If 50% of the volume of the present continental crust was formed by about 2,500 Myr ago it is possible that the continents have been growing more or less linearly during geological history<sup>33</sup>. There is increasing evidence, however, that the growth of the present-day continents, both in terms of surface area and thickness, was probably almost complete by 2,500 Myr ago (see below).

The model strontium isotope curve for the evolution of seawater shows a marked increase about 2,600 Myr ago, which is consistent with a major stage of crustal fractionation at that time<sup>33</sup>.

The factors of continental area and thickness, and the evidence of early (Archaean) crustal thickening and of early (late Archaean) emergence of continents above sea level, are interrelated. This interdependence is well illustrated by the conclusions of Wise<sup>36</sup> of freeboard (elevation of continents with respect to sea level) as a function of time: (1) the continents have had at least

90% of their present thickness for a minimum of 2,500 Myr. (2) There has been isostatic balance (constant freeboard) between oceanic and continental crusts throughout the Proterozoic and Phanerozoic, that is continental and oceanic volumes and areas have not changed significantly in this time span.

This picture is supported in particular by Watson's<sup>19</sup> conclusion about the lack of vertical movement of Archaean cratons for the last 2,000 Myr, and by the fact that the present thickness of continental crust in belts reworked at different times throughout the last 2,500 Myr is constant (38–40 km range) and independent of age<sup>37</sup>.

Isostatic modelling<sup>1</sup> shows that a 30-km thick crust can support ideally mountains that rise 2 km above sea level. Because there is widespread evidence that the continental crust had reached such a thickness by the late Archaean, it can be supposed that there were 2 km high mountains at that time in greenstone belt country—a likely result of vertical movement at a rate of 0.27 km Myr (ref. 17)—and even higher mountains above granulite-gneiss belts. Erosion of such elevated continents may account for the high proportion of immature sediments (arkoses and greywackes) in late Archaean greenstone belt sequences, the recycling of which during later geological time contributed to more mature detrital sediments (shales, sandstones and quartzites)<sup>38,39</sup>.

In Phanerozoic time continental fragmentation was accompanied by transgressions, and continental assembly by regressions<sup>40</sup>; it has long been known that the periods immediately following the formation of the Caledonian, Hercynian and Alpine fold belts were characterised by regressions<sup>40</sup>. In the mid-late Archaean the most intense period of 'orogenesis' and continental growth in earth history most likely resulted in the assembly of a large number of small plates (because of a greater ridge and subduction zone length) into more extensive, thick and stable plates by the early Proterozoic<sup>15,16</sup>. Because regression follows orogeny and accompanies continental assembly, the Archaean-Proterozoic boundary probably marks a period of profound regression followed, in the early Proterozoic, by widespread transgression that led to the formation of thick carbonate banks (for example, Coronation and Mount Isa Geosynclines) and miogeoclines (for example, Huronian Supergroup and the Western Labrador Trough).

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- Hargraves, R. B. *Science* **193**, 363–371 (1976).
- Wells, P. R. A. *Contr. Mineral. Petrol.* **56**, 229–242 (1976).
- Condie, K. C. *Plate Tectonics and Crustal Evolution* 288 (Pergamon, Oxford, 1976).
- O'Hara, M. J. *J. geol. Soc. Lond.* (in the press).
- Tarney, J. & Windley, B. F. *J. geol. Soc., Lond.* (in the press).
- James, D. E. *Bull. geol. Soc. Am.* **82**, 3325–3346 (1971).
- Windley, B. F. & Smith, J. V. *Nature* **260**, 671–675 (1976).
- Bridgwater, D., McGregor, V. R. & Myers, J. S. *Precamb. Res.* **1**, 179–197 (1974).
- Coward, M. L., Lintern, B. C. & Wright, L. in *The Early History of the Earth* (ed. Windley, B. F.) 323–330 (Wiley, London, 1976).
- England, P. C. & Richardson, S. W. *J. geol. Soc., Lond.* (in the press).
- Windley, B. F. & Davies, F. B. (in preparation).
- Tarney, J., Dalziel, I. W. D. & de Wit, M. J. in *The Early History of the Earth* (ed. Windley, B. F.) 131–146 (Wiley, London, 1976).
- Burke, K., Dewey, J. F. & Kidd, W. S. F. in *The Early History of the Earth* (ed. Windley, B. F.) 113–129 (Wiley, London, 1976).
- Salop, L. *Precambrian of the Northern Hemisphere* 378 (Elsevier, Amsterdam, 1977).
- Burke, K. C. A. & Dewey, J. F. in *Implications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) 2, 1035–1045 (Academic, London, 1973).
- Windley, B. F. *The Evolving Continents* 385 (Wiley, London, 1977).
- Hunter, D. R. *Precamb. Res.* **1**, 295–326 (1974).
- Watson, J. V. *Phil. Trans. R. Soc. A280*, 629–640 (1976).
- Card, K. D. et al. *Geol. Ass. Can. Spec. Pap.* **11**, 335–380 (1972).
- Hoffman, P. *Phil. Trans. R. Soc. A273*, 547–581 (1973).
- Davidson, A. *Geol. Ass. Can. Spec. Pap.* **11**, 381–434 (1972).
- Morgan, W. C. *Geol. Surv. Can. Pap.* **74**, 54, 42 (1975).
- Jackson, G. D. & Taylor, F. C. *J. Can. Earth Sci.* **9**, 1650–1669 (1972).
- Young, G. M. *Palaeogeog. Palaeochim. Palaeocol.* **7**, 85–101 (1970).

Table 2 Estimated percentages of continental crust formed by the late Archaean

Isotopes	% of present-day continents	Time by which % was formed	Ref.
Pb-U	Most	2,500	30
Pb & Sr	Almost all	2,500	31
Rb-Sr	Almost all	2,700–2,500	32
Rb-Sr	67	2,700–1,800	33
Rb-Sr	50	2,900–2,500	28



25. Douglas, R. J. W. & Price, R. A. *Geol. Ass. Can. Spec. Pap.* **11**, 625–688 (1972).
26. Gibb, R. A. *Earth planet. Sci. Lett.* **27**, 378–382 (1975).
27. Kearey, P. *Earth planet. Sci. Lett.* **28**, 371–378 (1976).
28. Moorbath, S. *Proc. Geol. Ass.* **86**, 259–279 (1975).
29. Moorbath, S., Welke, H. & Gale, N. H. *Earth planet. Sci. Lett.* **6**, 245–256 (1969).
30. Patterson, C. C. & Tatsumoto, M. *Geochim. cosmochim. Acta* **28**, 1–22 (1964).
31. Armstrong, R. L. *Rev. Geophys.* **6**, 175–199 (1968).
32. Jahn, B. M. & Nyquist, L. E. in *The Early History of the Earth* (ed. Windley, B. F.) 55–76 (Wiley, London, 1976).
34. Muehlberger, W. R., Denison, R. E. & Lidiak, E. G. *Am. Ass. Petrol. Geol. Bull.* **51**, 2351–2380 (1967).
35. Kroner, A. *Precamb. Res.* **4**, 163–214 (1977).
36. Wise, D. U. in *The Geology of Continental Margins* (eds Burk, C. A. & Drake, C. L.) 45–58 (Springer, Berlin, 1974).
37. Condie, K. C. *Bull. geol. Soc. Am.* **84**, 2981–2992 (1973).
38. Ronov, A. B. *Geochem. Int.* **1**, 713–737 (1964).
39. Veizer, J. *Contr. Mineral. Petrol.* **38**, 261–278 (1973).
40. Valentine, J. W. & Moores, E. M. *Nature* **228**, 657–659 (1970).

## Alcohol retards visual recovery from glare by hampering target acquisition

ADAMS and Brown<sup>1</sup> showed that relatively small doses of alcohol produced “large, significant, dose-related increases in the time required to recover foveal contrast sensitivity following bright light exposure”. As this effect of alcohol may be directly related to industrial and to car driving safety<sup>2</sup>, we set out to ascertain its precise origin. Adams and Brown concluded tentatively that the alcohol exerted its effect at the retinal level. The experiments presented here, however, implicate non-retinal mechanisms, as alcohol delays recovery from glare only when observers have difficulty localising or fixating the test stimulus.

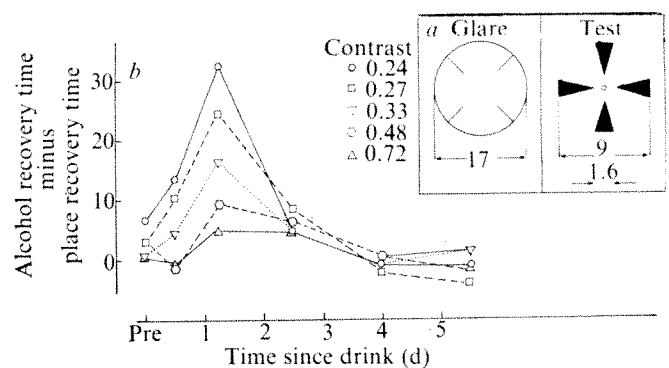
The first part of our study was a replication of the Adams and Brown experiment. Our subjects were four male college students with normal (6/6) or corrected-to-normal visual acuity. On separate days subjects ingested either alcohol (1.0 ml per kg body weight) or placebo. The two treatments were given in random order. The alcohol dose was 95% ethanol diluted 2:1 with fruit drink so that the total volume (ml) was three times the subject's weight (kg). The placebo was an equivalent volume of fruit drink alone. The drink was consumed in 20 min from a paper cup containing two ice cubes. In addition, 2 drops each of ethanol and eucalyptus oil were placed on the lid of the cup to minimise olfactory cues to the presence of alcohol<sup>1</sup>.

Details of stimulus presentation are given in Fig. 1. Subjects practiced the detection task to achieve stable performance at the beginning of each experimental session; they then were tested before drinking, and 30, 75, 150, 240, and 330 min after drinking. At each of these times, three sets of measurements were taken with 5 min allowed between sets to allow complete recovery from the adapting luminance level. The session always started in the early afternoon, and subjects were instructed to eat lunch beforehand.

As expected, alcohol produced large, significant increases in the time required to recover foveal contrast sensitivity. In particular, as Fig. 1 shows, the difference between recovery times with and without alcohol is maximum at the sampling time 75 min after ingestion and declines thereafter, and is most pronounced at low target contrasts. Analysis of variance (ANOVA) indicates that the depicted interaction between dose, time, and contrast level is statistically significant ( $P < 0.001$ , d.f. = 20, 60).

A simple but trivial explanation of the results of Adams and Brown and of our replication would be that following alcohol ingestion observers become more cautious about reporting the visibility of the test target, thereby spuriously increasing recovery times<sup>3,4</sup>. We decided therefore to use a methodology<sup>5</sup> which could discriminate alcohol-induced changes in sensitivity from changes in criterion (that is, willingness to report).

Using the same apparatus as before but without a glare source, we tested seven male college students. On half of the 2-s trials the small flickering test spot actually was presented; on the remaining trials no spot was presented. The subject's task was to respond either ‘yes’ (a test spot had been presented) or ‘no’. With each subject, 500 trials were run at various spot



**Fig. 1** *a*, The stimulus configuration of the  $8.6 \times 10^4$  cd m<sup>-2</sup> glare field on the left and the test field on the right. The 10 min arc test spot was located at the centre of the test field reference markers on a background of luminance 24.8 cd m<sup>-2</sup>. In the actual experiment, the glare field was located directly above the test field, which was at eye level. At the beginning of each measurement session, the subject fixated with his left eye the centre of the circular adapting (glare) field. The right eye was covered with a patch, and fixation was aided by four thin diagonally-orientated reference lines. Immediately after a 60-s exposure to the high intensity glare source, and with the eye-patch still in place, the subject looked directly below (straight ahead) and fixated the centre of the test field on which a test spot was presented intermittently (125-ms flashes at 4 Hz). The contrast of the test spot was under the experimenter's control. When the subject indicated orally that he saw the spot at the highest contrast level, the experimenter rotated a filter wheel one notch, reducing the target contrast a further step below the subject's threshold. When the subject's contrast sensitivity had recovered enough to restore the test spot to detectability, the spot contrast was reduced again. The time taken to recover to each of five predetermined contrast levels (0.72, 0.48, 0.33, 0.27, and 0.24) was obtained. These levels were chosen to give approximately equal intervals between each recovery point. *b*, Interaction of alcohol, contrast, and time for the first experiment. Each point is the mean difference (s) between recovery time with alcohol and recovery time with the placebo. Data from four subjects.

contrasts. Subjects were tested either following ingestion of alcohol (dose identical to that used before) or following ingestion of the placebo. Intermittent tests were carried out 30–140 min after ingestion, when glare recovery was most seriously retarded in our original experiment. The conditional probabilities of ‘yes’ responses in the presence of a test spot and ‘yes’ responses in the absence of a test spot were used to estimate<sup>5</sup> the observer's response criterion ( $\beta$ ). Analysis of variance indicated no significant alcohol-induced changes in  $\beta$  ( $P > 0.50$ ).

With response bias eliminated as an explanation, we sought to identify the source of the alcohol-induced delay in glare recovery times more precisely. Four new subjects participated in an experiment identical in every respect to our first, except that three sets of eye movement measurements (before, during, and after exposure to the glare) were taken every 15 min for 135 min following the 20-min drinking period. Standard electrooculographic<sup>6</sup> (EOG) methods produced records of eye position as a function of time. The resolution of our recordings was approximately one degree of visual angle.

With or without alcohol, very little eye movement occurred either before or during exposure to the glare source. But immediately after the 60 s of high intensity glare, there were many eye movements as if subjects ‘hunted’ for the flashing spot within the test field; after alcohol ingestion the eye movements were larger and more frequent. These eye movement patterns suggested that alcohol might combine with glare to prolong recovery time by impeding the subject's attempts to localise and fixate the target. Localisation would be hampered if alcohol ingestion were to disturb the subject's memory of precisely where the target would appear. Steady fixation would be hampered if alcohol were to impair control of the extraocular muscles<sup>7</sup>. Irregular visual fixation could produce enough

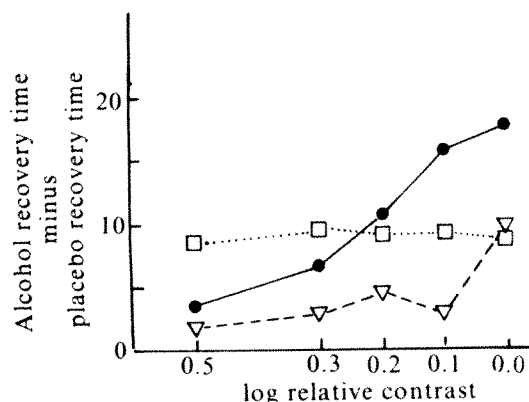
uncertainty about the target's spatial location to reduce its detectability<sup>8</sup>.

Three of the four subjects who participated in our original study were available for two additional experiments. In the first, the test spot locus was circumscribed by a small, dark circle, 12.5-arc min diameter. The circle was of high contrast, and clearly visible immediately after glare exposure even though the flashing test spot within was not. As a result, the subject would not have to remember where the target might appear thereby aiding target localisation. Details of the experimental design and procedure were as in the original experiment, except that the test spot luminance was three times that of the spot in the original experiment. In a second experiment, the test spot was enlarged (100-arc min diameter) to fill the entire area between the arrow reference marks (Fig. 1). Consequently, no matter where between the arrows the subject looked, his fixation would fall on some part of the flashing test spot. Enlarging the area of the test spot reduced its maximum luminance; to compensate, we reduced the background luminance to 0.89 cd m<sup>-2</sup>. In both these experiments, as before, glare recovery was measured with spots whose contrasts covered a range of 0.5 log units; a single set of five measurements was made before, and 30, 90, 150 and 210 min after drink ingestion.

As shown by Fig. 2, the effect of alcohol on recovery from glare was greatly reduced when either the small test spot was circumscribed by a clearly defined circle or the test spot was enlarged. With the enlarged test spot, no effects involving alcohol were statistically significant (ANOVA, all  $P > 0.5$ ). This is what would be expected if the alcohol-induced retardation of glare recovery in our original experiment had been partly caused by an unsteadiness of fixation which the enlarged test spot rendered unimportant. The contrast independent elevation shown in Fig. 2 is not statistically significant ( $P > 0.80$ ) and is due to greatly increased recovery times for just one subject. With the small, circumscribed test spot, one effect involving alcohol (the interaction of alcohol and contrast) was significant ( $P < 0.03$ , d.f. = 4, 8), due mainly to the increased recovery time at the lowest contrast level. This reduced effect of alcohol would be expected if in our original experiment alcohol had made subjects unsure about the location of the target and if the easily seen, high-contrast circle in the present experiment had made them less unsure. The small residual effect of alcohol may reflect difficulty in maintaining precise fixation following alcohol consumption, a problem that would be less critical with the enlarged spot.

Our experiments confirm that ingestion of alcohol causes a

**Fig. 2** Mean difference (s) between recovery time with alcohol and recovery time with the placebo. Data are shown for all five contrast levels and for all three glare experiments. The data have been normalised in both this figure and in all analyses of variance to produce 47.52-s average recovery time for each subject (the average for the original experiment). Only the data from those subjects who participated in all three experiments are shown. Data shown were collected at those times following alcohol ingestion which should have produced the largest effect of alcohol: 75 min for the original experiment (●), 90 min for the large spot (□), and 90 min for the circumscribed spot (△).



loss of visual sensitivity following glare. But non-retinal processes are primarily responsible for the effect of alcohol on glare recovery. We emphasise that the non-retinal origin of this alcohol-induced visual disability does not lessen its potential for adversely affecting driving and related visual tasks. On the contrary, uncertainty about the location<sup>8</sup> of possible targets imposes an inescapable reduction of visual sensitivity of any driver; if alcohol either retards a driver's target acquisition<sup>9</sup> or causes him to mislocalise the target<sup>10</sup>, visibility necessarily will be reduced and his ability to respond to the target impaired. Moreover, we cannot rule out the possibility that in driving, the effect of alcohol that we studied can sum with other, previously identified perceptual consequences of alcohol ingestion<sup>11</sup>.

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- Adams, A. J. & Brown, B. *Nature* **257**, 481-483 (1975).
- Moskowitz, H. & Murray, J. T. *J. Stud. Alcohol* **37**, 40-45 (1976).
- Tong, J. E., Knott, V. J., McGraw, D. J. & Leigh, G. Q. *J. Stud. Alcohol* **35**, 1003-1022 (1974).
- von Wright, J. M. & Mikkonen, V. *Scand. J. Psychol.* **11**, 167-175 (1970).
- Green, D. M. & Swets, J. A. *Signal Detection Theory and Psychophysics* (Wiley, New York, 1966).
- Young, L. R. & Sheena, D. *Behav. Res. Meth. Instr.* **7**, 397-429 (1975).
- Wilkinson, I. M. S., Kime, R. & Purnell, M. *Brain* **97**, 785-792 (1974).
- Cohn, T. E. & Lasley, D. J. *J. opt. Soc. Am.* **64**, 1715-1719 (1974).
- Adams, A. J., Brown, B., Flom, M. C., Jones, R. T. & Jampolsky, A. *Am. J. Optom. Physiol. Optics* **52**, 729-735 (1975).
- Greenhouse, D. S., Cohn, T. E. & Stark, L. presented at *Mtg Ass. Res. Vis. Ophth.* Sarasota, Florida (April 1977).
- Moskowitz, H. & Sharma, S. *Human Factors* **16**, 174-180 (1975).

## Visual discrimination between small objects and large textured backgrounds

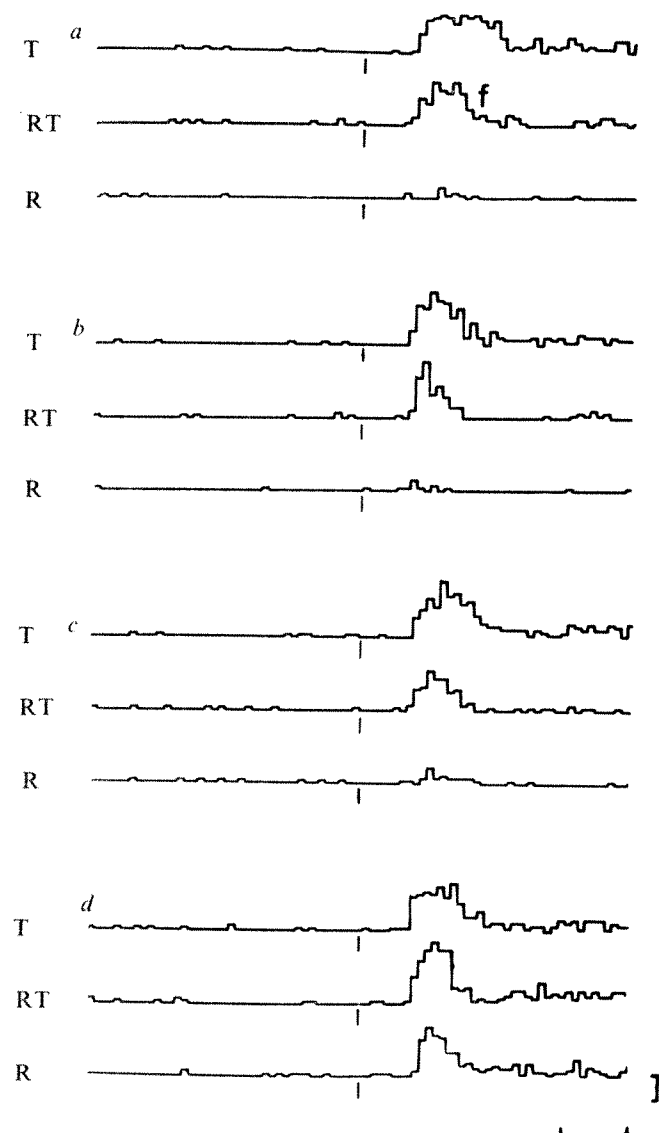
THE remarkable ability of certain higher order insect visual neurones to discriminate between movement of a small object or target and a large textured background has been clearly demonstrated by Palka<sup>1,2</sup> and O'Shea and Fraser-Rowell<sup>3</sup>. In addition, movement of a large textured background such as a windmill pattern is inhibitory to detection of motion of a small target by this neurone, known as the descending contralateral movement detector (DCMD). The response of this identifiable neurone to motion of small objects is inhibited by rotation of the windmill pattern over a specific range of high spatial frequencies of the pattern, but is augmented by low spatial frequencies in the pattern. For this neurone we have quantitatively determined the spatial frequency at which the effect of the windmill pattern becomes inhibitory.

The DCMD is a visual interneurone in the ventral nerve cord which is excitatory to motor neurones involved in jumping. It originates in the protocerebrum where it receives excitatory input through an electrical synapse from the LGMD (lobular giant movement detector) which has a dendritic field in the shape of a fan across the entire lobula. The LGMD receives at least two kinds of inhibitory inputs: lateral inhibition on neurones peripheral to the fan of dendrites, and inhibitory input proximal to the convergence of the fan of dendrites (post-convergence inhibition)<sup>3,4</sup>. The post-convergence inhibition samples activity across the entire visual field but is effective only for higher velocities<sup>3,4</sup> of patterns than used in these experiments.

Responses of the DCMD in *Schistocerca gregaria* were obtained by conventional methods<sup>2</sup>. The receptive field of the DCMD is approximately a hemisphere, and motion detection



does not depend on the direction of motion<sup>5,6</sup>. The region of greatest sensitivity lies along the equator posterior to the animal's transverse plane, and it is primarily this region which was tested in these experiments. Two visual stimuli were presented. One, an approximately 5° (in visual angle subtended at the cornea) light disk which is occluded by a moving edge, yields a large sustained train of spikes over the entire time



**Fig. 1** Sets of PST histograms demonstrating inhibition of small target response by radial grating pattern. This set of histograms is to be viewed in sequential groups of three traces. R refers to rotation of the radial grating pattern only, T refers to motion of the edge in the small disk only, and RT refers to simultaneous radial grating pattern rotation and edge motion. Radial gratings are of 90 black and white stripes of equal angular width (a), 40 stripes (b), 20 stripes (c) and 10 stripes (d). This figure shows, for 90, 40 and 20 stripes, that the excitatory response to the motion of the small target alone (traces T) precedes the inhibition due to the simultaneous radial grating rotation (traces RT). In the second trace from the top (a), marks inhibition occurring at the falloff of response to small target motion. Patterns and disk are adjacent along the equator with disk posterior, and are 9 inches from the cornea; the radial grating subtends 37° visual angle and the disk 5.6°. Time of initiation of motion is shown by small vertical bar near centre, below each trace, so that spontaneous activity is seen to the left; motion terminates at end of record. Each trace represents 17 trials, and the T and RT are interleaved with 30 s interstimulus interval (ISI). The R are a separate run with 30 s ISI. Experiment of 21 February 1976, light surround (see Fig. 2); order of T/RT was 90, 20, 10, 40 stripes; order of R was 10, 40, 20, 90; time bar 100 ms; vertical scale 59 spikes per s; sustained baseline is 0 spikes per s.

course of motion of the edge. The other, a large circular radial grating pattern (windmill pattern) of an adjustable number of alternating black and white stripes of equal width which is rotated about its centre, yields a burst of spikes only on initiation of rotation regardless of velocity.

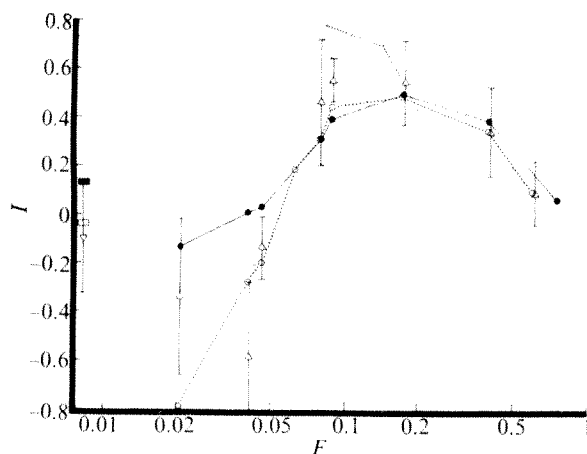
In Fig. 1 the post-stimulus time (PST) histograms are shown for a typical experiment to illustrate the nature of the inhibition in time. For these experiments the clearest distinction between small objects and large backgrounds can be made in the spatial frequency domain. The spatial frequency spectrum of an intensity pattern is given by the Fourier transform of that pattern. This calculation shows that the magnitude of the spatial frequency spectrum of the radial grating pattern has a minimum sensible spatial frequency of approximately  $0.85F$  and peak spectrum spatial frequency of  $1.09F$ , where  $F$  is the single number spatial frequency geometrically calculated as  $1/L$ ,  $L$  being the angular distance subtended by one stripe cycle at the outermost edge of the pattern. In Fig. 2 this geometric value of  $F$  has been used, which represents the 25% of peak spectrum point. (Note: in ref. 7, page 336, the value  $0.85F$  erroneously reads  $1.3F$ , and  $1.09F$  erroneously reads  $1.64F$ .)

The spatial frequency components of the moving edge in the small disk are predominantly at low spatial frequencies, which is complementary to the situation for the radial grating pattern.

In Fig. 2 the inhibition  $I$  of the response to the small target caused by the radial grating pattern is plotted as a function of  $F$ , the nominal minimum spatial frequency of the radial grating. Since the curve is positive for  $F$  greater than 0.05 cycles per degree the radial grating is inhibitory when all of its spatial frequency components lie above 0.05 cycles per degree. When any of its components lie below 0.05 cycles per degree the radial grating becomes excitatory to the response to the small target, as the curve there is negative. The peak of the curve lies at about 0.2 cycles per degree which is thus approximately the spatial frequency of maximum inhibition. The curve falls to zero at 1.0 cycles per degree because that is the maximum resolvable spatial frequency for this animal<sup>7</sup>. Thus low spatial frequencies are excitatory and high spatial frequencies are inhibitory to the response to low spatial frequencies, as long as the high spatial frequencies are resolved. In Fig. 2 a further difference in the DCMD responses elicited by high and low spatial frequency stimuli is greater variability to low excitatory spatial frequencies than to high ones. At lower spatial frequencies fewer ommatidia are stimulated by a moving pattern so that higher integrative units have either fewer inputs to average, or are more susceptible to their inherent noise.

A peaked inhibition curve like that of Fig. 2 can be obtained with radial grating pattern diameters down to a minimum of 30° (visual angle). Inhibition could occasionally be found at 0.2 cycles per degree for radial grating pattern diameters down to a minimum of 10°. The conclusion is that the radial grating patterns become inhibitory backgrounds at the optimal inhibitory spatial frequency if their diameter exceeds 10°, but for inhibition to occur over a measurable range of spatial frequency their diameter must exceed 30°. The inhibition is not uniformly dependent on the distance between the radial grating pattern and the small target. The inhibition is also not dependent on grating rotation velocity below about 300° (pattern plane angle) per s.

A model for the observation that stimuli with predominantly high spatial frequency components are inhibitory to those with low ones can be based on optic lobe electrophysiology any neuroanatomy of locust<sup>8,9</sup> and dipterans<sup>10,11</sup>. Arnett<sup>10</sup> had shown that movement of a small contrasting pattern can be detected by first order interneurons (monopolar cells), and lateral inhibition has been demonstrated in locust optic lobe by Horridge *et al.*<sup>8</sup>, and O'Shea and Fraser-Rowell<sup>3,4</sup>. A detector of high spatial frequencies can be derived by spatial summation over the entire visual field of monopolar cell outputs, and a detector of primarily low spatial frequencies can be derived by spatial summation over the entire field of another set of monopolar cells having lateral inhibitory connections among



**Fig. 2** Inhibition ( $I$ ) of response to motion in small disk plotted as a function of the lower limit,  $F$  (cycles per degree visual angle subtended at cornea), of spatial frequency content of the radial grating pattern. Inhibition  $I$  is defined as  $(NE - NI)/NE$ , where  $NE$  is the number of spikes in the DCMD in response to motion in small disk alone, and  $NI$  is the number of spikes in response to simultaneous motion in disk and rotation of the radial grating pattern. When the number of spikes in response to simultaneous motion  $NI$  is greater than  $NE$ , inhibition  $I$  becomes negative and is, in fact, excitation. In all experiments, these two kinds of trials are interleaved to increase stability of the measurement. ●, Average of means of continuous series of 14 experiments 12 December 1975–14 February 1976 in completely dark surround. ○, Average of means of continuous series of three experiments in light surround, 17–21 February 1976. Light continuous dotted line, negative of response to rotation only of radial gratings from data of acuity studies of 1972<sup>2</sup> to show acuity limits inhibition at high frequencies. △, Data of one experiment (21 February 1976) in light surround; bars indicate  $\pm$  the sum of standard error of  $NE$  and  $NI$ ; each point is the mean of 34 or 68 trials with 30-s ISI and interleaved presentation of disk motion trials with simultaneous disk motion and radial grating rotation. ■, Average of active controls on dark surround experiments. □, Average of active controls on light surround experiments. ▽, Active control on single light surround experiment. (An active control is rotation of the unilluminated radial grating with disk motion.) Stimulus parameters: luminance of a brighter bar of radial grating,  $1,200 \text{ lm m}^{-2}$ ; dark bar,  $\sim 0 \text{ lm m}^{-2}$ ; small disk has luminance equivalent to bright bar of radial grating; light surround luminance approximately  $1.5 \log_{10}$  units below that of bright bar; when the disk is occluded in light surround, the occluded portion has the luminance of the surround, but the excitatory response is approximately the same as in dark surround. The radial grating tangential velocity is  $11^\circ$  (visual angle) per s for most runs of this figure, or  $36^\circ$  per s in pattern plane angle. The velocity of translation of the occluding edge is  $12^\circ$  (visual angle) per s, and the light disk subtends  $5.6^\circ$  (visual angle); the radial grating subtends  $37^\circ$  (visual angle) for all but a few runs of this figure. In all experiments except as noted the disk and grating pattern are directly adjacent along the equator.

themselves. In the locust the latter summation would take place as excitatory synapses at or before the fan of LGMD dendrites in the lobula<sup>4</sup> via interneurons from the medulla, and the former summation as inhibitory synapses from the first set of monopolars on to these interneurons in the medulla. Since the LGMD drives the DCMD directly, this model gives for DCMD response two separate channels selectively responding to high and low spatial frequencies respectively, with an inhibitory cross-connection from the high frequency channel to the low frequency channel. Further tests of these ideas require probing of more peripheral units than the LGMD.

In vertebrate vision the reduction of retinal sensitivity during relative motion of retina and image is known as saccadic suppression. The suppressive or inhibitory effect on human vision (measured with a low spatial frequency test flash) produced by relative motion of gratings<sup>12,13</sup> is parallel to the above results for the locust DCMD. It is of interest that the locust visual system processes high and low spatial frequency image information in the same manner as do some perceptual

channels of the human visual system.

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1. Palka, J. *J. exp. Biol.* **50**, 723–732 (1969).
2. Palka, J. *Am. Zool.* **12**, 497–505 (1972).
3. O'Shea, M. & Fraser-Rowell, C. H. *Nature* **254**, 53–55 (1975).
4. Fraser-Rowell, C. H., O'Shea, M., Williams, J. L. D. *J. exp. Biol.* **68**, 157–185 (1977).
5. Palka, J. *J. Insect Physiol.* **13**, 235–248 (1967).
6. Kien, J. in *The Compound Eye and Vision of Insects* (ed. Horridge, G. A.) 410–422 (Oxford University Press, Oxford, 1975).
7. Palka, J. & Pinter, R. B. in *The Compound Eye and Vision of Insects* (ed. Horridge, G. A.) 321–337 (Oxford University Press, Oxford, 1975).
8. Horridge, G. A., Scholes, J. H., Shaw, S. & Tunstall, J. in *The Physiology of the Insect Central Nervous System* (eds Treherne, J. E. & Beament, J. W. L.) 165–202 (Academic London, 1965).
9. Meinertzhagen, I. *Phil. Trans. R. Soc. B.* **274**, 555–596 (1976).
10. Arnett, D. W. *J. Neurophys.* **35**, 429–444 (1972).
11. Strausfeld, N. J. & Campos-Ortega, J. A. *Science* **195**, 894–897 (1977).
12. Brooks, B. A. & Fuchs, A. F. *Vision Res.* **15**, 1389–1398 (1975).
13. Mateef, S., Yakimoff, N. & Mittrani, L. *Vision Res.* **16**, 489–492 (1976).

## Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley

RESERVES of assimilate present in wheat and barley crops at flowering, and available for later translocation to the grains, could buffer grain yield against environmental stresses during grain filling. This so-called pre-anthesis assimilate contribution to grain yield can be expressed as a percentage of yield ( $P_1$ ). Archbold<sup>1</sup>, and later Thorne<sup>2</sup>, concluded that  $P_1$  was small, being no more than 20%. But only one result (12% for irrigated wheat at Cambridge<sup>3</sup>) refers to a crop in the field as distinct from plants in pots, and no studies considered the effect of stress during grain filling. Recently Gallagher *et al.*<sup>4,5</sup> reported substantial contributions:  $P_1$  averaged 43% over six crops of wheat and barley at Nottingham; this amounted to more than 300 g per m<sup>2</sup> of dry material in two crops and, in the severe drought of 1970, 39% of total dry matter present at anthesis. They assumed, with some supporting evidence from one barley crop<sup>6</sup>, that the pre-anthesis contribution was given by the decrease from anthesis to maturity in dry weight of non-grain parts of the crop. *In situ* labelling with <sup>14</sup>CO<sub>2</sub> of the whole crop canopy at frequent intervals before and after anthesis would seem to be the least equivocal way of estimating  $P_1$ . Using this method we have determined  $P_1$  in wheat and barley. It averaged only 12% (watered crops) and 22% (droughted crops), and did not agree with estimates for the same crops obtained by the method of Gallagher *et al.*<sup>4,5</sup>.

Wheat and barley were grown during 1974–75 at the Centro de Investigaciones Agrícolas del Noroeste (CIANO) near Ciudad Obregon in north-west Mexico. There was no rain and crops were either flood irrigated frequently (I) or only until 27th January, about 40 d before anthesis, creating a drought (D) regime. Weather and general experimental procedures were as described elsewhere<sup>7</sup>. Grain yields for regime I were high (Table 1). Significant differences between D and I in leaf water potential arose about 10 d before anthesis and then increased steadily. There were substantial reductions in yield in regime D, especially for the wheat cultivars, which were later in flowering (Table 1).

The canopy labelling with <sup>14</sup>CO<sub>2</sub> began on 20 February and was repeated on separate 1.08 m<sup>2</sup> portions of the crops every 7–14 d until near maturity. The proportion of total above ground <sup>14</sup>C at maturity found in the grain was slightly greater for any given date of labelling in regime D, but was remarkably consistent between cultivars and species (Fig. 1). The relationship of Fig. 1a agrees with results<sup>2,8,9</sup> obtained in non-crop conditions.

Combining the data of Fig. 1 with information on changes in above ground dry weight,  $P_1$  was estimated to be 12–13% under

frequent irrigation, rising to 27% for droughted wheat (Table 2). But in absolute terms (grain dry weight,  $GDW_1$  in  $g\ m^{-2}$ ), it was similar for regimes I and D. As a proportion of total dry weight at anthesis,  $GDW_1$  amounted to 8.5% (I) and 11.5% (D). Considering that  $GDW_1$  for barley included the husk, already present at anthesis and amounting to 8% (I) and 9% (D) of final grain weight, the true contribution of stored pre-anthesis assimilate was clearly lower for barley than for wheat.

The method used to estimate  $P_1$  in Table 2 assumes that any contribution of carbon from roots, or variation in the proportion of carbon to dry matter in the total crop compared with that in the grain, or fluctuations in crop growth rate within the two key periods, are of minor importance. On the other hand, neglect of the loss of tissue, formed before anthesis, and lost during the post-anthesis period could be important. Such losses seem to be substantial under irrigation, probably due to the saprophytic decay of lower early-formed, and hence unlabelled, leaves. A loss of  $100\ g\ m^{-2}$  would seem probable (R.A.F., unpublished) under the I regime, and correcting  $\Delta TDW_2$  ( $TDW$ , total dry weight) for this would increase  $GDW_2$  by  $75\ g\ m^{-2}$  and lower mean  $P_1$  to 11%. We have also assumed that no assimilate formed before the date of first labelling was later transferred to the grain. Inspection of Fig. 1 shows this to be incorrect, but the small total dry weights at first labelling mean the amounts of assimilate neglected are small. The transfer to the grain of assimilate formed as early as 27 d before anthesis (Fig. 1a) may be associated with the transfer of leaf nitrogen in the form of protein.

Considering this correction to  $GDW_2$  under regime I, the values of  $GDW_1 + GDW_2$  agree closely with the observed grain yield (Table 2). Also the figure of 13% for irrigated wheat is similar to that of 12% for an irrigated wheat crop determined with  $^{14}CO_2$  by Lupton<sup>3</sup>, and to values which can be calculated with our procedure from the extensive results of Birecka and Dakic-Wlodkowska<sup>8</sup>, albeit obtained with wheat grown in pots. An independent upper estimate of the contribution of pre-anthesis assimilate in the case of adjacent irrigated crops of Yecora 70 at CIANO is given by the quantity of sugar stored in stems at anthesis. This amounted to  $50\ g\ m^{-2}$  (15% stem sugar;  $320\ g\ m^{-2}$  stem tissue), to which should be added leaf protein transfer to the grain, for these purposes set equal to the decrease, anthesis to maturity, in leaf lamina dry weight, amounting to  $40\ g\ m^{-2}$  ( $200\ g\ m^{-2}$  of green leaf lamina tissue, 20% decrease in dry weight excluding leaf loss). The total available material thus estimated ( $90\ g\ m^{-2}$ ) agrees well with the value of  $GDW_1$  of Table 2 ( $69\ g\ m^{-2}$ ).

Table 2 makes possible a separate estimate of  $P_1$  based on the decline in non-grain dry weight after anthesis ( $\Delta TDW_2$  less

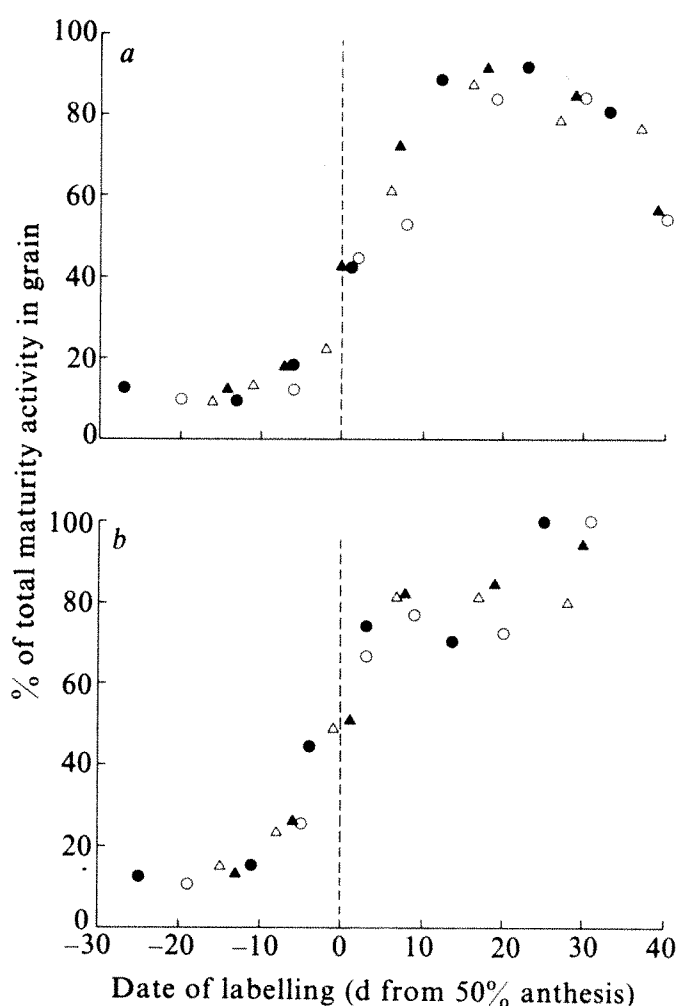


Fig. 1 The fraction at maturity of total crop  $^{14}C$  found in the grain as a function of the time (d from anthesis) when the  $^{14}CO_2$  was assimilated; a, frequently watered, (I); b, droughted, (D); Yecora 70 (●), Ciano (▲), CM67 (○) and WI 2198 (△).  $^{14}CO_2$  assimilation involved placing a clear polyethylene chamber over  $1.08\ m^2$  of crop. Into the chamber  $100\ \mu Ci$  of  $^{14}CO_2$  was introduced. The chamber remained closed and was stirred with a fan for 10 min. Labelling was carried out within 3 h of noon. At maturity, a  $0.3\ m^2$  quadrat was cut from each region previously labelled and the specific activity of oven dried subsamples were determined by combustion, trapping of the  $^{14}CO_2$  in a liquid scintillation mixture and counting.

Table 1 Grain yield and yield components in the 1974–75 experiment at CIANO, Mexico

Species	Cultivar	Date of 50% anthesis	Grain yield ( $g\ m^{-2}$ )	Kernel weight (mg)
Frequently watered throughout (I)				
Wheat	Yecora 70	March 17	639	45
	Ciano 67	March 10	563	43
Barley	CM 67	March 4	609	49
	WI2198	March 6	610	54
Droughted during grain filling (D)				
Wheat	Yecora 70	March 15	293	30
	Ciano 67	March 9	293	32
Barley	CM 67	March 3	363	38
	WI 2198	March 5	349	40
s.e.m.			18	0.5

Plots were sown on 14 December 1974 and received moderate fertilisation ( $75\ kg\ ha^{-1}\ N$  and  $18\ kg\ ha^{-1}\ P$ ). A split plot design (main plot = water regime, sub plot = cultivar) with three replications was used. Each sub plot was  $15\ m \times 1.8\ m$ .

observed  $GDW$ ), as proposed by Gallagher *et al.*<sup>4,5</sup>. But the calculation suggests practically zero contribution in all situations of Table 2. This has been confirmed for irrigated wheat by other experiments with many cultivars at CIANO over several years (ref. 10 and R.A.F., unpublished). The apparent discrepancy when compared to the  $^{14}C$  results of Table 2 probably arises because the absence of net change in the non-grain dry weight after anthesis, does not preclude translocation of pre-anthesis assimilate to the grain, balanced by the accumulation of post-anthesis assimilate in non-grain parts. The fact that the fraction of  $^{14}C$  in the grain from post-anthesis labelling averaged only 0.78 (Fig. 1, Table 2) supports this explanation. Also sampling in other irrigated wheat crops at CIANO showed that from anthesis to maturity the dry weight of the peduncle and chaff increases, while that of the leaves and the remainder of the stem decreases. If these latter decreases are taken to reflect assimilate translocated to the grain, the contribution agrees reasonably well with the 13% value in Table 2.

Our results point to a smaller value of  $P_1$  than that reported by Gallagher *et al.*<sup>4,5</sup>. Notwithstanding the discussion of the preceding paragraph, it is likely that in many situations the method of these authors overestimates  $P_1$ , because the dry

**Table 2** Estimation of the proportion of grain yield derived from pre-anthesis assimilate ( $P_1$ )

Variable	Frequently watered		Droughted	
	Wheat	Barley	Wheat	Barley
Pre-anthesis period				
Start date*	24	15	23	14
Start TDW†	282	410	282	410
Anthesis TDW	760	750	610	655
$\Delta TDW_1$	478	340	328	245
$F_1$	0.14	0.19	0.24	0.27
$GDW_1$	64	65	79	67
Post-anthesis period				
Maturity TDW	1,358	1,367	888	1,038
$\Delta TDW_2$	598	617	278	383
$F_2$	0.74	0.75	0.78	0.83
$GDW_2$	445	465	215	317
Overall				
$GDW_1 + GDW_2$	509	530	294	384
$P_1$	13%	12%	27%	17%
Observed GDW	601	609	293	356

TDW, total dry weight ( $\text{g m}^{-2}$ ); GDW, grain dry weight ( $\text{g m}^{-2}$ ). TDW measured by harvests ( $0.30 \text{ m}^2$  quadrat per sub plot) at dates corresponding to those when  $^{14}\text{C}$  labelling was carried out, and by harvest ( $1.80 \text{ m}^2$  per sub plot) at maturity; anthesis TDW estimated by interpolation, and the increase in total dry weight during the pre-anthesis ( $\Delta TDW_1$ ) and post-anthesis ( $\Delta TDW_2$ ) periods was calculated.  $F_1$  and  $F_2$  are the average proportions of total crop  $^{14}\text{C}$  at maturity in the grain for labelling in the pre- and post-anthesis periods, respectively, and were obtained from Fig. 1. The contribution to grain yield of pre-anthesis ( $GDW_1$ ) and post-anthesis ( $GDW_2$ ) assimilate in absolute terms ( $\text{g m}^{-2}$ ) are equal to  $F_1 \times \Delta TDW_1$ , and  $F_2 \times \Delta TDW_2$ , respectively. The pre-anthesis assimilate contribution as a percentage ( $P_1$ ) is given by  $100 \times GDW_1 / (GDW_1 + GDW_2)$ . The data were averaged for the two cultivars of each species.

\*Time (d) before anthesis of first  $^{14}\text{C}$  labelling date.

†Average of both water regimes used.

weight change in non-grain parts includes tissue, principally leaves, lost due to decay, wind and so on. At other sites in Mexico,  $P_1$  estimated with this method for various non-droughted wheat cultivars ranged from 12 to 98%<sup>11</sup>, and clearly increased as site rainfall and disease incidence increased. We believe this reflects tissue loss, which even at CIANO with no rain or disease amounted to at least  $100 \text{ g m}^{-2}$ . Consequently the results from Nottingham<sup>4,5</sup> may have overestimated the importance of the pre-anthesis contribution of assimilate to grain yield. Even in the most suitable conditions for the expression of this contribution in our experiment (well grown wheat crops, post-anthesis drought reducing kernel weight 30%), it amounted to only 27% of final yield or  $79 \text{ g m}^{-2}$  according to the  $^{14}\text{C}$  data.

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1. Archbold, H. K. *Nature* **156**, 70–73 (1945).
2. Thorne, G. N. *Rep. Rothamsted exp. Stn.* **1973** **2**, 5–25 (1974).
3. Lupton, F. G. H. *Ann. appl. Biol.* **64**, 363–74 (1969).
4. Gallagher, J. N., Biscoe, P. V. & Scott, R. K. *J. appl. Ecol.* **12**, 319–36 (1975).
5. Gallagher, J. N., Biscoe, P. V. & Hunter, B. *Nature* **264**, 541–2 (1976).
6. Biscoe, P. V., Gallagher, J. N., Littlejohn, E. J., Monteith, J. L. & Scott, R. K. *J. appl. Ecol.* **12**, 295–318 (1975).
7. Fischer, R. A. & Laing, D. R. *J. agric. Sci., Camb.* **87**, 113–22 (1976).

8. Birecka, H. & Dakic-Wlodkowska, L. *Acta Soc. Bot. Poloniae* **35**, 637–62 (1966).
9. Rawson, H. M. & Evans, L. T. *Aust. J. Agric. Res.* **22**, 851–63 (1971).
10. Aguilar, I. & Fischer, R. A. *Agrociencia (Mexico)* **21**, 185–98 (1975).
11. Midmore, D. J. thesis, Univ. Reading (1976).

## Physiological energetics of cock-crow

ALTHOUGH much is known about bird song in the behavioural context there is no exact information concerning the efficiency of the communication system itself in terms of energy usage. Little attention has been paid to study of energy relationships in animal communications. The domestic fowl (*Gallus domesticus*) is a convenient subject on which to make physiological measurements of the energy involved in sound production, because of its size and its readiness to make calls in the laboratory, and because of the sheer volume of sound it produces. I have made such measurements and report them here.

Cock-crow is visibly and audibly one of the most vigorous and spectacular forms of avian vocalisation. The maximum root-mean-square (r.m.s.) sound pressure level attained during the performance is equivalent to a value of 100 dB at a distance of 1 m (Fig. 1a). This corresponds to a peak value of 103 dB. This very impressive achievement is accompanied by enormous rises in air-sac pressure and air flow in the trachea (Fig. 1c, d). Pressure rises to  $60 \text{ cm H}_2\text{O} = 6 \times 10^4 \text{ dyn cm}^{-2}$  and averages  $55 \text{ cm H}_2\text{O} = 5.5 \times 10^4 \text{ dyn cm}^{-2}$ . Air flow reaches  $500 \text{ ml s}^{-1}$  and averages  $\sim 350 \text{ ml s}^{-1}$  during the cycle.

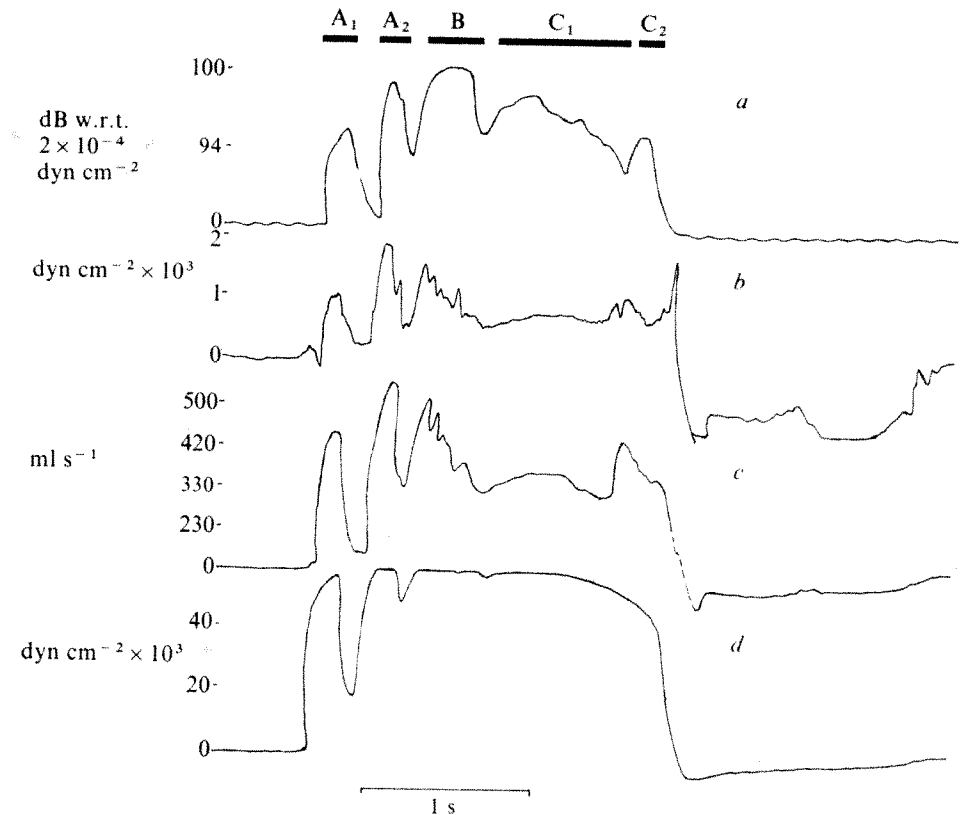
Compared with a normal expiration, these values represent increases of  $\sim 100$ -fold and 15-fold for pressure and flow respectively. This indicates that, concomitant with sound production, there is a rise in airway resistance of some 6–7-fold. This rise is attributed to a change in the configuration of the syrinx, the sound-producing organ, involving, amongst other things, a narrowing of the syringeal lumen by the intruding tympaniform membranes<sup>1–3</sup>. In keeping with this idea, Fig. 1b indicates that the pressure changes in the trachea are comparatively small and, therefore, that virtually the whole of the pressure head generated in the air sacs is spent during the passage of air through the syrinx.

The fluid energy losses in the syrinx are accounted for mainly by frictional dissipation, but also to a lesser extent by conversion of a part of the energy into the mechanical vibration of the external tympaniform membranes, and hence into airborne vibrations. It is possible to estimate approximately the efficiency of this conversion process, and therefore of the effectiveness of the lung air sac-syrinx apparatus as an audio-generator, by comparing the total fluid energy losses incurred by the effort with the amount of sound energy finally produced. The work performed in expelling air from the lung air sac system to the atmosphere is estimated by measuring graphically the area enclosed by the pressure-volume curve during a single crowing cycle (Fig. 2d). The volume curve is itself obtained by graphical integration of the linearised flow curve. The final figure is  $\sim 3.5 \text{ W s}$ . The duration of the crow is 2 s, so the average work rate is 1.75 W. This does not, however, represent total crowing effort since no account has been taken of the internal work of the expiratory muscles lost as contraction heat, and the external work done in moving the viscera enclosing the air sacs.

To calculate the sound energy radiated, the sound pressure level curve given in Fig. 1a is first re-drawn as an equivalent power curve ( $0 \text{ dB} = 2 \times 10^{-4} \text{ dyn cm}^{-2} = 10^{-19} \text{ W cm}^{-2}$ ) which is then integrated over the crowing cycle to yield the total energy per  $\text{cm}^2$  (Fig. 3). The resultant value, assuming a maximum r.m.s. sound pressure level of 100 dB, works out at  $\sim 9 \times 10^{-7} \text{ W s}$ . This is equivalent to an average sound reading throughout the cycle of 95 dB. The total area



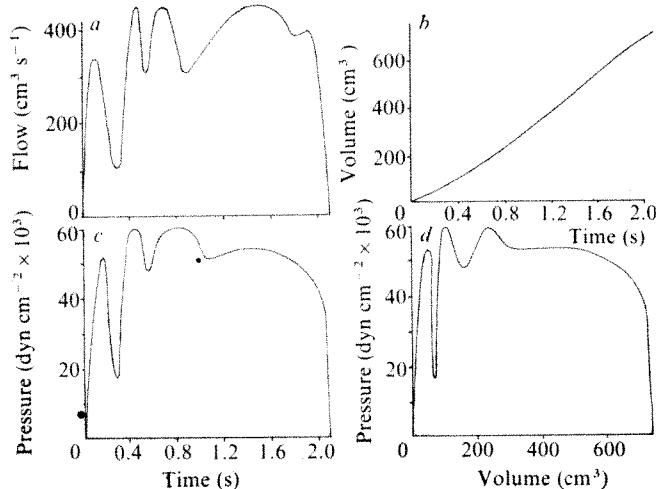
**Fig. 1a**, Average sound pressure level, at a distance of 1 m, during crow. Recordings were made with a Brüel and Kjaer 4161 omnidirectional microphone connected to a Nagra 1V SJ recorder and levels were confirmed by a Brüel and Kjaer 2209 precision sound level meter. Recordings were passed through a Grass 7P3 fast integrator to give a display of the sound pressure waveform. Bars above indicate division of crowing cycle into sections: A<sub>1</sub>, A<sub>2</sub>, introductory; B, transitional; C<sub>1</sub>, plateau; C<sub>2</sub>, final kick. **b**, Pressure changes in trachea. **c**, Air-flow rate. The flow meter was implanted into the trachea 2 cm caudal to the larynx. The calibration is nonlinear. **d**, Pressure changes in interclavicular air sac. Flow and pressure measurements were made with Grass PT5 manometers. Scale bar, 1 s.



over which the sound is radiated is calculated approximately by assuming, as is done in the case of human speech<sup>4</sup>, that the sound isobars are arranged in front of the mouth as concentric hemispheres. At a distance of 1 m, the total area of such a hemisphere is  $\sim 6 \times 10^4 \text{ cm}^2$ ; therefore, the total energy radiating from the mouth is  $\sim 9 \times 10^{-7} \times 6 \times 10^4 \text{ W s} = \sim 0.054 \text{ W s}$ . The average sound power is therefore  $0.054/2 = 0.027 \text{ W}$  or 27 mW. This figure is approximately 27 times the maximum speech power radiated during very loud human conversation<sup>5</sup>.

The efficiency of the sound-producing process, measured as the ratio of sound to fluid power, is  $0.055/3.5 \times 100 = 1.6\%$ . The overall efficiency, given in terms of the ratio of sound output to total physical exertion measured by the energy consumption of the driving muscles, is less than

**Fig. 2** Calculation of efficiency of sound production process. **a**, Linearised flow wave form. **b**, Volume curve obtained by integration of (a). **c**, Air-sac pressure waveform. **d**, Air-sac pressure-volume curve obtained from (c) and (b). The area enclosed by the curve gives the fluid work done during a single crowing cycle, given by  $\int PdV = \text{work}$ .

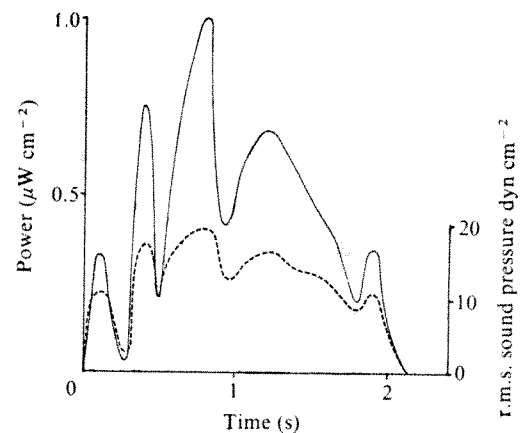


1.6% since it also includes on the input side thermodynamic energy wastage in the contracting tissue and energy spent moving the viscera.

Songbirds are capable of generating sounds which seem to be much louder in proportion to their body size, and therefore in proportion to their power resources, than chickens (unpublished observations). The efficiency of the sound-producing process in these smaller birds therefore seems to be greater. This may be related to well-known differences in the structure of the syrinx<sup>6</sup>, particularly the assemblage of intrinsic muscles which the chicken syrinx altogether lacks, and which seems to be responsible for accurately regulating both the shape of the syrinx and the flow of air through it.

I know of no other estimates of the efficiency of sound production of birds. It is, however, interesting to compare the figures for the chicken with those given for certain insects which use an entirely different mechanism of sound production, based on the rubbing of one part of the body against another. Crickets of the family Gryllinae can pro-

**Fig. 3** Power curve (—), obtained from sound pressure curve (---). The total energy per  $\text{cm}^2$  is obtained by measuring the area beneath the power curve.



duce  $6 \times 10^{-2}$  mW of sound with an overall efficiency of 5% whilst the mole cricket *Gryllotalpa vineae* produces 1.2 mW with a remarkable efficiency of 30% (ref. 7). These insects are clearly more economical in their use of energy than the fowl in which the overall efficiency is probably no greater than 1%. Nothing definite can be said as to the position of songbirds on the efficiency scale, although it seems to be greater than 1%.

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1. Brackenbury, J. H. *J. Zool., Lond.* (in the press).
2. Gaunt, A. S., Stein, R. C. & Gaunt, S. L. *J. exp. Zool.* **183**, 241–262 (1973).
3. Gaunt, A. S., Gaunt, S. L. & Hector, D. H. *Condor* **78**, 208–223 (1976).
4. Sacia, C. F. *Bell Syst. Tech. J.* **4**, 627 (1925).
5. Dunn, H. K. & Farnsworth, D. W. *J. acoust. Soc. Am.* **10**, 184 (1939).
6. Warner, R. W. *J. Zool., Lond.* **168**, 381–393 (1972).
7. Bennet-Clark, H. C. *Nature* **234**, 255–259 (1971).

## Regeneration of endoderm by ectoderm isolated from mouse blastocysts

CELL determination can be described as the progressive restriction in developmental options during embryogenesis<sup>1</sup>. Mouse embryo cells, in particular, remain undetermined for a relatively long period, and the fate of cells seems to be labile throughout the period of cleavage<sup>2–4</sup>. Micro-surgical analysis of mouse blastocysts, however, has shown that the developmental potential of inner cell mass and trophoblast cells becomes restricted during blastocyst formation, so that neither of these early cell types retains the option of differentiating into the other<sup>5–7</sup>. We have now investigated whether the primary ectoderm, which forms in the inner cell mass on day 5 of gestation<sup>8,9</sup>, retains the option of differentiating into endoderm. We found that when ectoderm was isolated from mouse blastocysts by immunosurgery<sup>10</sup> it could regenerate an outer layer of endoderm during further culture. The capacity of isolated ectoderm to differentiate into endoderm depended both on the initial mass of the embryo and on the time that the ectoderm was isolated from the inner cell mass.

Viable ectoderm cells can be isolated from pre-implantation mouse embryos by immunosurgery of blastocysts and thin inner cell masses<sup>11</sup>. Ectoderm thus isolated and cultured on feeder layers for 1–22 d differentiates into mesodermal and ectodermal derivatives, although it does not reform visceral endoderm<sup>12</sup>. But because the development of the inner cell mass in intact blastocysts requires a critical mass of tissue<sup>13</sup>, we investigated the developmental potential of ectoderm with increased mass isolated from aggregation chimaeras.

The survival of ectoderms isolated from chimaeric embryos and incubated in our culture conditions depended strongly on the number of embryos used to form the chimaera (Table 1). Ectoderms isolated from single embryos and chimaeras composed of two embryos degenerated; most of the ectoderms from chimaeras composed of four embryos survived, although only a few of these regenerated an endoderm layer. Most ectoderms isolated from chimaeras composed of eight or 10 embryos, however, survived and regenerated an outer layer of endoderm cells (Table 1, Fig. 1a, b). These cells had the characteristic apical vacuoles and microvilli of visceral endoderm cells (Fig. 2a, b) of mouse embryos *in vivo*<sup>8,17</sup> and *in vitro*<sup>8,18</sup>.

Although isolated ectoderms seemed by phase contrast microscopy to be essentially free of contaminating endoderm cells, it was possible that some endoderm cells

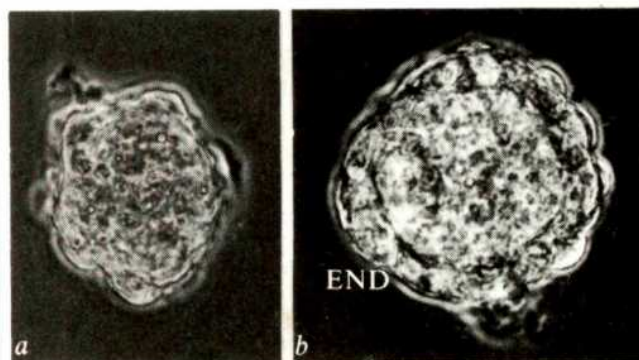


Fig. 1 Regeneration of endoderm by ectoderm isolated from chimaeric mouse blastocysts. *a*, Ectoderm just after immunosurgery of isolated inner cell mass (24 h after late blastocyst stage) (phase contrast,  $\times 310$ ). *b*, Ectoderm cultured for 48 h after immunosurgery. END, endoderm layer. (Phase contrast,  $\times 310$ .)

survived immunosurgery and rapidly proliferated to form a new endodermal layer. To determine whether regenerated endoderm arose from contaminating endoderm cells, we labelled cells after the second immunosurgery with <sup>3</sup>H-thymidine, and compared the grain counts per nucleus in ectoderm and regenerated endoderm after 48 h of culture (Table 2). The dilution of <sup>3</sup>H-thymidine labelling indicates a turnover time of about 24 h for the DNA of these cells. Assuming that there is little re-utilisation of salvaged DNA<sup>19</sup>, these results indicate a cell-cycle time for both ectoderm and endoderm cells *in vitro* of approximately 24 h. Therefore there would have to be a substantial number of contaminating endoderm cells (about 24) to produce the mean number of endoderm cells (96) present at 48 h (Table 2). We serially sectioned nine ectoderms fixed just after immunosurgery and found an average of three (0,0,1,1,2,2,3,8 and 10) contaminating endoderm cells for each ectoderm. Even these few endoderm cells may not have survived, because isolated ectoderms commonly lost a few dead outer cells during the 24 h following immunosurgery. We conclude that there were too few viable contaminating endoderm cells to account for the final number of regenerated endoderm cells and that both the ectoderm and the endoderm present at the end of the experiment arose from the ectoderm cell population.

The time at which ectoderm was isolated from the

Table 1 Effect of number of embryos in chimaeric blastocysts on endoderm regeneration by isolated mouse ectoderm cells

No. of embryos in chimaera	Total no.	No. of degenerated ectoderms	Alive, no endoderm	Endoderm + ectoderm
2	14	14	0	0 (0%)
4	18	5	11	2 (11%)
8	19	1	7	11 (57.9%)
10	31	2	6	23 (74.2%)

Two-cell embryos were obtained from mated Dub:(ICR) mice (Flow Laboratories) after superovulation with pregnant mares' serum gonadotropin and human chorionic gonadotropin. They were cultured in modified standard egg culture medium as before<sup>13</sup>. At the four- to eight-cell stage, between two and 10 embryos were aggregated to form chimaeras in the presence of phytohaemagglutinin<sup>14</sup>. At the late blastocyst stage (96-h culture from the two-cell stage) inner cell masses were isolated by immunosurgery<sup>10</sup>, embryos were incubated in rabbit anti-mouse L-cell (NCTC Clone 929) serum (1:1 dilution for 25 min) and guinea pig complement (Gibco) (1:5 dilution for 45 min). Ectoderms were isolated by further immunosurgery after inner cell masses had been cultured for another 24 h in modified Eagle's basal medium<sup>15,16</sup> containing 5% foetal calf serum and 5% newborn calf serum. Ectoderms were cultured individually for 48–72 h in drops of medium, with or without paraffin oil, in bacteriological Petri dishes (Falcon) to prevent attachment and outgrowth of ectodermal cells on the substrate. They were scored for the presence of an endodermal layer under the dissecting microscope ( $\times 140$ ).



**Table 2** Grain counts on nuclei of  $^3\text{H}$ -thymidine-labelled cells before and after endoderm regeneration

Cell type	Hours cultured	No. of samples	Mean no. of cells per sample*	Mean % labelled	Mean no. of grains per nucleus $\pm$ s.e.m.
Ectoderm	0	2	292	74	$43.8 \pm 2.4$
	24	3	192	86	$17.7 \pm 1.4$
	48	2	253	67	$9.2 \pm 1.5$
Endoderm	24	1	109	61	$15.9 \pm 1.7$
	48	3	96	73	$9.1 \pm 2.6$

Two-cell embryos were cultured overnight and made into chimaeras (10 embryos each) as described in Table 1. Inner cell masses were obtained by immunosurgery from these embryos at the late blastocyst stage; ectoderms were obtained from inner cell masses after 24 h. Ectoderms were labelled with  $^3\text{H}$ -thymidine ( $10^{-2}$   $\mu\text{Ci ml}^{-1}$ , specific activity 45 Ci  $\text{mmol}^{-1}$ ) for 4 h, washed and cultured for the designated time before hypotonic treatment and fixation in glacial acetic acid and 95% ethanol (1:1) in watch glasses essentially as described<sup>20</sup>. At these concentrations of  $^3\text{H}$ -thymidine there was no effect on cell viability<sup>25</sup>. Autoradiographic exposure was for 3 d. Endoderm and ectoderm layers were separated before fixation by enzyme treatment as before<sup>21</sup>.

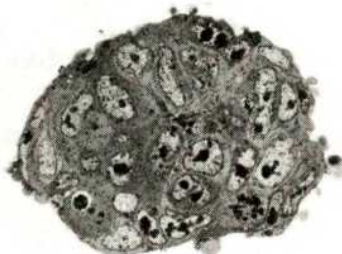
\*These numbers may be underestimates because of losses during separation or fixation.

inner cell mass by immunosurgery also strongly influenced its ability to regenerate endoderm. When ectoderms were isolated from inner cell masses 24 or 48 h after isolation of the inner cell masses from late blastocysts the ectoderms regenerated a layer of endoderm. But virtually all ectoderms isolated 72 h after isolation of inner cell masses from late blastocysts failed to regenerate endoderm, and instead they degenerated within 48 h of further culture (Table 3).

Our results show that the inner cells of the isolated

**Fig. 2** Thick section of ectoderm before and after endoderm regeneration. *a*, Ectoderm fixed and sectioned just after immunosurgery (24 h after late blastocyst stage) (bright field,  $\times 430$ ). *b*, Ectoderm cultured for 48 h after immunosurgery. Note complete outer layer of endoderm and central cavity containing cell debris (bright field,  $\times 430$ ). Ectoderms were isolated from chimaeric blastocysts as described in Table 2. Samples were fixed in glutaraldehyde and osmium and embedded and sectioned as before<sup>9</sup>.

*a*



*b*



mouse inner cell mass—the embryonic ectoderm—retain the capacity to differentiate *in vitro* into endoderm for a period well beyond the time that endoderm differentiation normally occurs. Because isolated ectoderms in our experiments regenerate visceral endoderm 48 h after the late blastocyst stage, there seems to be a period of at least 2 d before ectoderm of the mouse inner cell mass undergoes any restriction in its developmental fate. Although failure of ectoderm to regenerate endoderm when isolated after 72 h of culture could imply that ectoderm itself is sensitive to the immunosurgery and culture procedures, we consider this possibility unlikely because of the high incidence of development when it is isolated at earlier stages (Table 3). We conclude that loss of the ability to form visceral endoderm is due to a determinative event between 48 and 72 h after the late blastocyst stage.

By contrast, the developmental potential of visceral endoderm cells seems already to be restricted at the late blastocyst stage. Endoderm cells that are transferred to host blastocysts colonise only the extra-embryonic regions, while ectoderm cells colonise the entire conceptus<sup>6</sup>. Also, visceral endoderm isolated from mouse and rat egg cylinders fails to survive when transferred to ectopic sites<sup>21-23</sup>. The definitive endoderm of the foetus therefore does not seem to arise from the visceral endoderm<sup>6,21-23</sup>.

The major difference between our approach and that of others who did not observe endoderm regeneration by isolated ectoderms<sup>11,12</sup> was our use of chimaeric embryos to increase the mass of the isolated tissues and our prevention of ectoderm attachment to substrate. We interpreted the disintegration of ectoderms isolated from single or paired embryos as an indication that survival of functional inner cells requires a critical mass of tissue which does not exist once trophoblast and endoderm cells are destroyed by immunosurgery. It may be possible, however, to circumvent this requirement in part by

**Table 3** Effect of ICM culture time on formation of endoderm by isolated ectoderms

Hours of culture of isolated ICM	Total no. of ectoderms	No. of degenerated ectoderms	No. of live ectoderms without endoderm	No. of ectoderms with endoderm
24	28	0	6	22
48	14	0	0	14
72	31	29	1	1

Two cell embryos were cultured overnight and chimaeras (10 embryos each) were formed. Inner cell masses (ICM) were isolated by immunosurgery from blastocysts (Table 1) and cultured for various times before isolation of ectoderm by immunosurgery. Growth was scored after 48 or 72 h. Data was pooled from three experiments.

providing a feeder layer of fibroblasts<sup>12</sup>. The failure of ectoderm even from chimaeras of 10 embryos to survive isolation after 72 h of culture implies either that endoderm has an essential, perhaps nutritional function at this time or that after losing the ability to form endoderm, ectoderm cells must attach to survive or to differentiate further. There did not seem to be any obvious morphological change in the ectoderm, however, that correlated with the loss of ability to regenerate endoderm after 72 h in culture. After 24 h of culture isolated inner cell masses have a homogeneous core of ectoderm cells; after 48 and 72 h, the ectoderm has developed into a pseudostratified columnar epithelium surrounding a proamniotic cavity. The cavity, however, increases from approximately 30  $\mu$ m to 100  $\mu$ m in diameter during this time<sup>26</sup> (and unpublished results).

In view of our results with mouse ectoderm isolated 24–72 h after the late blastocyst stage, it seems that the ability to form visceral endoderm, a trait shared by clonal embryonal carcinoma cells<sup>24</sup>, is lost at a time (7–8 equivalent days of gestation) when the embryonic ectoderm can still develop into all three definitive germ layers when it is transferred to ectopic sites<sup>22,23</sup>.

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- Hadorn, E. *Brookhaven Symp. Biol.* **18**, 148–161 (1965).
- Hillman, N., Sherman, M. I. & Graham, C. J. *Embryol. exp. Morph.* **28**, 263–278 (1972).
- Gardner, R. L. & Rossant, J. in *Embryogenesis in Mammals* 5–18 (Elsevier, Amsterdam, 1976).
- Kelly, S. J. in *The Early Development of Mammals* (eds Balls, M. & Wild, A. E.) 97–105 (Cambridge University Press, Cambridge, 1975).
- Gardner, R. L. *J. Embryol. exp. Morph.* **28**, 279–312 (1972).
- Gardner, R. L. & Papaioannou, V. in *The Early Development of Mammals* (eds Balls, M. & Wild, A. E.) 107–132 (Cambridge University Press, Cambridge, 1975).
- Rossant, J. *J. Embryol. exp. Morph.* **33**, 979–990 (1975); **33**, 991–1001 (1975).
- Snell, G. D. & Stevens, L. C. in *Biology of the Laboratory Mouse* 2nd edn (ed. Green E. L.) 205–245 (McGraw Hill, New York, 1966).
- Wiley, L. & Pedersen, R. A. *J. exp. Zool.* **200**, 389–402 (1977).
- Solter, D. & Knowles, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5099–5102 (1975).
- Strickland, S., Reich, E. & Sherman, M. I. *Cell* **9**, 231–240 (1976).
- Hogan, B. & Tilly, R. *Nature* **265**, 626–629 (1977).
- Snow, M. H. L. *J. Embryol. exp. Morph.* **35**, 81–86 (1976).
- Goldstein, L. S., Spindle, A. I. & Pedersen, R. A. *Radiat. Res.* **62**, 276–287 (1975).
- Mintz, B., Gearhart, J. D. & Guymont, A. O. *Devl Biol.* **31**, 195–199 (1973).
- Spindle, A. I. & Pedersen, R. A. *J. exp. Zool.* **186**, 305–318 (1973).
- Solter, D., Damjanov, I. & Skreb, N. Z. *Anat. Entwickl. Gesch.* **132**, 291–298 (1970).
- Solter, D., Biczysko, W., Pienkowski, M. & Koprowski, H. *Anat. Rec.* **180**, 263–280 (1974).
- Cleaver, J. E. *Thymidine Metabolism and Cell Kinetics* 203–207 (North-Holland, Amsterdam, 1967).
- Tarkowski, A. K. *Cytogenetics* **5**, 394–400 (1966).
- Levak-Svajger, B. & Svajger, A. *Experientia* **27**, 683–684 (1971); *J. Embryol. exp. Morph.* **32**, 445–459 (1974).
- Diwan, S. B. & Stevens, L. C. *J. natn. Cancer Inst.* **57**, 937–942 (1976).
- Grobstein, C. J. *J. exp. Zool.* **119**, 355–379 (1952).
- Martin, G., Wiley, L. M. & Damjanov, I. *Devl Biol.* (in the press).
- Kelly, S. J. & Rossant, J. *J. Embryol. exp. Morph.* **35**, 95–106 (1976).
- Whiley, L. M., Spindle, A. I. & Pedersen, R. A. *Devl Biol.* (in the press).

## Non-metastasising variants selected from metastasising melanoma cells

THE cell surface may be involved in the metastatic process of cancer cells<sup>1</sup>. To determine whether membrane properties influence or reflect the metastasising capacity, it is essential to have metastasising and non-metastasising variants of the same tumour. Lectins which bind specifically to surface carbohydrates<sup>2</sup> have been used successfully as selective agents to obtain cells with membrane carbohydrate alterations<sup>3–5</sup>. We

Table 1 *In vitro* characterisation of melanoma variants

Cells	Selection	Surviving colonies*	%Agglutination†	% WGA binding‡
F-1	—	—	97	100
Wa-2	2nd	10	85	80
Wa-3	3rd	50	66	69
Wa-4	4th	> 200	24	62

\*Surviving colonies growing out from  $2 \times 10^6$  cells following incubation for 10 d in selective medium (100  $\mu$ g ml<sup>-1</sup> WGA in Eagle's minimal essential medium supplemented with 10% foetal calf serum) and 2 weeks in normal medium without WGA.

†No. of agglutinated cells/no. of total cells  $\times$  100% following incubation of  $1 \times 10^6$  cells per ml with 10  $\mu$ g WGA per ml for 5 min at room temperature<sup>7</sup>.

‡F-1 control cells (100% binding) bound 275.5 c.p.m. per  $\mu$ g cell protein after 5 min of incubation of  $1 \times 10^6$  cells per ml with 5  $\mu$ g <sup>125</sup>I-WGA per ml at 4 °C (specific activity  $4.8 \times 10^4$  c.p.m. per  $\mu$ g WGA—supplied by A. Rapin).

have selected variants of melanoma cells for surface differences in using toxic concentrations of lectins in the hope that such variants would exhibit different metastasising capacities. We report here the isolation of variants from metastasising melanoma cells resistant to toxic concentrations of wheat germ agglutinin (WGA). These variants show reduced to a loss of metastasising capacity and altered surface properties.

Mouse B-16 melanoma cells (adapted to grow in tissue culture<sup>6</sup>; supplied by Dr I. J. Fidler, designated as F-1) were maintained in tissue culture for 10 d in selective medium containing WGA at 100  $\mu$ g ml<sup>-1</sup>. The WGA-containing medium was then replaced with normal medium and the surviving cells were allowed to grow into discrete colonies in the ensuing 2–3 weeks. All of the resulting six individual colonies were isolated as clones. One of these six clones was further subjected to the same selection procedure successively to produce first Wa-2, then Wa-3 and Wa-4 clones. Table 1 shows that with each additional selection, more cells survived the selective pressure. This increased resistance to the toxic effects of WGA was clearly confirmed in Fig. 1 which compares the growth rates of the parental line F-1 and one of the Wa-4 lines in different concentrations of WGA.

The metastasising capacity was tested by injecting  $5 \times 10^4$  cells intraperitoneally (i.p.) and scoring the resulting tumours in various organs 2–3 weeks later. All animals developed peritoneal tumour masses with ascites and died within 2–4 weeks of injection. Parental cells metastasised in 92% of the animals to mediastinal and mesenteric lymph nodes, and in 58% to liver, kidneys and lungs, as shown in Table 2. In contrast, Wa-2 cells showed a greatly decreased metastasising capacity, while Wa-3 and Wa-4 cells failed to metastasise at all.

Tumorigenicity was tested by injecting  $2 \times 10^5$  cells intravenously (i.v.) and 2–3 weeks later examining the tumour nodules in the lungs, generally the only site where tumours developed after i.v. injection. With successive WGA selections, the metastasising capacity of the variants decreased before tumorigenicity was reduced (Wa-2) while tumorigenicity began to decrease only at the point where no metastases could be detected (Wa-3 and Wa-4) (Table 2). This decreased tumorigenicity was further confirmed by injecting the tumour cells subcutaneously to eliminate the possible complicating factors affecting the survival of circulating tumour cells. Preliminary experiments showing the same pattern in nude mice rule out the possibility that the decreased tumorigenicity was simply due to an alteration in histocompatibility antigens.

Substantial differences in growth rates could influence the results of assays for tumorigenicity and metastasising capacity. But, although *in vivo* growth rates have not yet been assessed, all cell lines showed similar growth rates in tissue culture, with generation times in the range 14–16 h (Fig. 1).

Some of the surface properties of the variants were shown to be different from the parental cells as reflected by a substantial decrease in agglutinability with WGA (Table 1). In addition,



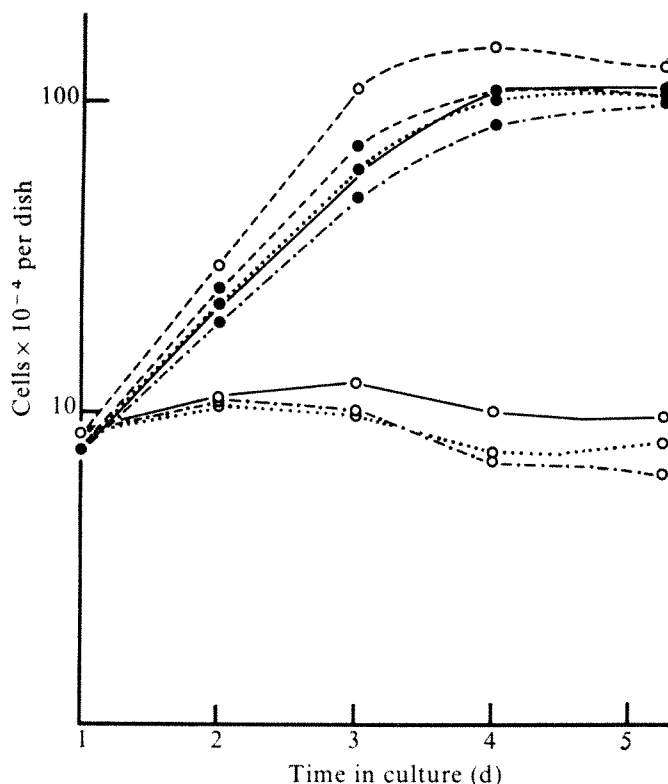


Fig. 1 Growth curves of parental and variant melanoma cell lines in the presence of WGA. Parental F-1 (○) and a Wa-4 variant (●) were grown in 0 (---), 33 (—), 50 (· · ·) and 75 μg WGA per ml (---) in Eagle's minimal essential medium with 10% foetal calf serum on 10 mm × 35 mm Falcon plastic culture dishes. WGA was added on day 1. Cells were removed daily with trypsin-EDTA and counted in Coulter counter. Each point represents the average value of duplicate dishes.

a decrease in lectin binding was also observed with some lines (Table 1).

The original metastasising line provided by Fidler and the completely non-metastasising variants described here display a maximal difference in metastasising capacity, while they exhibit no significant difference in growth rates *in vitro*. A detailed study of such lines should shed some light on the basic mechanisms underlying the process of metastasis.

By successively passing the B-16 cells through the lungs of the animals, Fidler<sup>8</sup> established lines showing a five- to tenfold increase in metastasising capacity which was defined in his experiment as the number of pulmonary nodules developed after i.v. injection of the tumour cells. Efforts to find surface differences with these lines have been rather disappointing<sup>9</sup>. This could have been because the quantitative difference in metastasising capacity may not have been large enough to allow the detection of biochemical alterations.

Comparison of the properties of metastasising and non-metastasising variants showed metastasising cells to be invasive,

where the non-metastasising variants were not invasive. The following preliminary observations were further noted. The non-metastasising variants displayed the greater homotypic adhesion. With successive WGA selection, there seemed to be a gradual increase in the organisation of some of the microstructural elements, from the parental line showing disorganised pattern to Wa-4 lines showing well organised stress fibres. Peptide patterns seen with slab gel electrophoresis following surface labelling were basically similar for the different cell types with the exception of one or two bands which were diminished in the non-metastasising variants.

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1. *Fundamental Aspects of Metastasis* (ed. Weiss, L.) (North-Holland, Amsterdam, 1976).
2. Sharon, N. & Lis, H. *Meth. Membr. Biol.* 3, 147–200 (Plenum, New York, 1975).
3. Gottlieb, C., Baenziger, J. & Kornfeld, S. *J. biol. Chem.* 250, 3303–3309 (1975).
4. Stanley, P., Narasimhan, S., Simionovitch, L. & Schachter, H. *Proc. natn. Acad. Sci. U.S.A.* 72, 3323–3327 (1975).
5. Meyer, A., Ungkitchanukit, A., Nairn, R. & Hughes, R. C. *Nature* 257, 137–139 (1975).
6. Fidler, I. J. *J. natn. Cancer Inst.* 45, 773–782 (1970).
7. Burger, M. M. *Meth. Enzym.* 32, 615–621 (1974).
8. Fidler, I. J. *Nature* 242, 148–149 (1973).
9. Nicolson, G. L. *et al.* in *Cell and Tissue Interactions* (eds Burger, M. M. & Lash, J.) (Raven, New York, 1977).

## Activation of complement by variant-specific surface antigen of *Trypanosoma brucei*

ANTICOMPLEMENT factors have been shown to occur in certain parasitic infections of man and animals. Hammerberg *et al.*<sup>1</sup> demonstrated the presence of a factor from *Taenia taeniaeformis* capable of activating complement through the alternate pathway. Similar findings have been reported for a substance in the cercarial coat of *Schistosoma mansoni*<sup>2</sup>. In trypanosomiasis, no such parasitic activity has been reported although hypocomplementaemia has been recognised as a consistent feature of the disease. Marked reductions in complement C4 and C3 were observed in infections with *Trypanosoma lewisi*<sup>3</sup>, *Tr. rhodesiense*<sup>4</sup> and *Tr. congolense*<sup>5</sup>. One possible mechanism is that the decrease of these complement components results from activation by antigen-antibody complexes. Deposits of immunoglobulins and complement have been demonstrated in kidneys of mice infected with *Trypanosoma brucei*<sup>6,7</sup> and monkeys infected with *Tr. rhodesiense*<sup>4</sup>. We present here evidence for an alternative mechanism operating through direct activation of human complement, via the classical pathway, by an isolated variant-specific surface antigen of *Tr. brucei*.

*Tr. brucei* grown in normal rats was isolated from infected blood using the method of Lanham<sup>8</sup>. In an initial experiment four preparations were used. The first fraction contained live trypanosomes; the second fixed parasites (1% formalin) and the third and fourth were 12,000g (30 min) supernatant and pellet fractions from trypanosomes sonicated on ice for 1 min. All these fractions were tested for the ability to activate human complement in a haemolytic assay<sup>9</sup>. The results showed that each of the four fractions depressed the provided CH<sub>50</sub> (CH<sub>50</sub>, 50% haemolytic dose) by at least 40% (Table 1). Decomplementation also occurred when bovine or rat serum was used as the source of complement. Similar supernatant and pellet fractions derived from *Tr. congolense* were also active (Table 1). Since these preparations may have contained antigen-antibody complexes, analogous fractions were derived from cloned organisms grown in lethally-irradiated mice (900 R) and tested as before. The

Table 2 Metastasising capacity and tumorigenicity of melanoma variants

Cells	Metastasising capacity*		Tumorigenicity†
	Lymph nodes	Other organs	
F-1	24/26	15/26	50/50
Wa-2	5/10	0/10	15/15
Wa-3	0/16	0/16	12/14
Wa-4	0/16	0/16	23/32

\*No. of animals showing metastases per total no. of animals tested following i.p. injection of  $5 \times 10^4$  cells. Lymph nodes: mediastinal and mesenteric. Other organs: liver, adrenal glands, kidneys and lungs in this order of frequency.

†No. of animals showing tumours per total no. of animals tested after injection of  $2 \times 10^5$  cells into the tail vein.

**Table 1** Ability of various preparations from *Tr. brucei* and *Tr. congolense* to depress complement activity

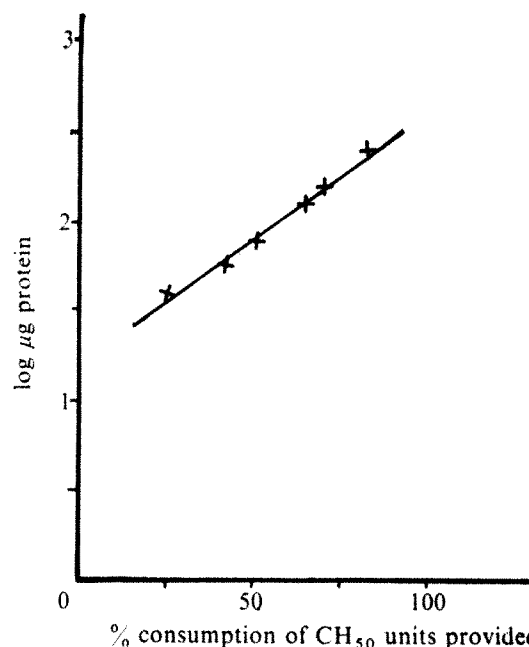
Parasite	Preparation	% Consumption of CH <sub>50</sub> per ml
<i>Tr. brucei</i>	Live	57
	Formalised	53
	Supernatant	41
<i>Tr. congolense</i>	Pellet	58
	Supernatant	47
	Pellet	52

Each preparation used in the assays was derived from  $4 \times 10^7$  parasites and contained in 0.2 ml of veronal-buffered saline. Human serum, obtained from normal donors, was adjusted to provide approximately 10 CH<sub>50</sub> per ml. One ml of the diluted serum was mixed with each fraction or buffer and incubated for 1 h at 37 °C. After incubation the particulate substances were removed by centrifugation and the residual complement titre (CH<sub>50</sub> per ml) determined according to the method of Rapp and Borsos<sup>9</sup>. Sheep cells, sensitised with rabbit haemolysin, were used at a concentration of  $2.5 \times 10^8$  per ml.

results were similar, indicating that the observed utilisation of complement was independent of immune complexes.

The results obtained using the supernatant fraction suggested that the variant antigen, a major soluble glycoprotein, could be involved. To examine this hypothesis a variant specific surface antigen from *Tr. brucei*, clone 052, was prepared using the method of Cross<sup>10</sup>. The parasites were grown from a stabilate in lethally-irradiated mice, followed by passage for 2–3 d in normal rats and then isolated. The purified glycoprotein had a *pI* of 6.9 and ran as a single band of 64,000 apparent molecular weight in sodium dodecyl sulphate (SDS)–7.5% polyacrylamide gels. These values are in agreement with published data<sup>10</sup>. No host proteins were detected in the preparation by immunodiffusion. This variant antigen, tested in a haemolytic assay as before, caused extensive de complementation using as little as 50 µg protein. A dose–response curve indicated that the de complementation was a logarithmic function of the amount of protein added (Fig. 1). Bearing in mind the extensive differences postulated among *Tr. brucei* surface antigens<sup>10</sup>, a second variant antigen from clone 055 was tested. This glycoprotein was also active.

A further series of experiments was performed to determine the mode of activation of complement. It has been shown by Fine *et al.*<sup>11</sup> that, since the classical and properdin pathways of complement activation have different cation requirements (calcium and magnesium for the former and only magnesium for the latter), EDTA and EGTA may be used to distinguish between the two. EDTA chelates both Ca<sup>2+</sup> and Mg<sup>2+</sup> ions while EGTA binds Ca<sup>2+</sup> effectively but has little affinity for Mg<sup>2+</sup>. In the following experiment, 270 µg of the purified antigen were incubated, for 1 h at 37 °C, with 1 ml of normal human serum (NHS, approximately 320 CH<sub>50</sub> per ml) made 10 mM with either EDTA or EGTA. The residual complement activity was measured as before. The results showed that there was no de complementation in the presence of these chelators, suggesting that activation of complement proceeds by the classical pathway. But, a particulate fraction (1.2 mg protein) prepared from the same clone and tested similarly, depressed complement activity by 72% in the presence of EGTA.



**Fig. 1** A sample of 1 ml of diluted normal human serum (10 CH<sub>50</sub> ml<sup>-1</sup>), was incubated with various amounts of the variant antigen, or buffer, for 1 h at 37 °C. The residual haemolytic titre (CH<sub>50</sub> ml<sup>-1</sup>) was determined using the method of Rapp and Borsos<sup>9</sup>.

This would indicate the presence, in the trypanosome, of another factor capable of activating the alternate pathway.

To confirm the results obtained using specific metal chelators, C1, C4, C2 and C3 activities were measured. The variant antigen was incubated with normal human serum and the residual activities of these individual components estimated using methods described by Rapp and Borsos<sup>9</sup>. The titres were calculated as the number of effective molecules per ml of the individual component being assayed<sup>9</sup>. The results showed preferential utilisation of C1, C4 and C2, with only a moderate change in C3, indicating classical pathway of activation (Table 2).

To test the ability of the variant antigen to cause permeability changes in the skin normal female rats, shaved over the dorsal region were given 0.5 ml of 1% brilliant blue R 10 min before intradermal inoculation of 20 µg of the variant antigen, histamine (5 µg), or buffer. Marked extravasation of dye occurred within 10 min at sites inoculated with variant antigen or histamine. Control sites showed only minimal extravasation of the dye at the site of needle trauma. Administration of an antihistamine (mepyramine maleate 30 mg per kg body weight), before the intradermal inoculations completely abolished the reaction to histamine and to variant antigen. These results suggested that variant antigen mediated production of anaphylotoxins had occurred.

Our observations indicate that complement activation by variant antigen, possibly released during the destruction of trypanosomes at each parasitaemic wave, might be an important contributory factor in depression of complement levels in man<sup>12</sup>

**Table 2** Consumption of some individual complement components in normal human serum incubated with a variant antigen of *Tr. brucei*

Component assayed	Effective molecules provided	Cellular intermediate	Source of other components	% consumption
C1	$7.4 \times 10^{12}$	EAC4hu*	C2hu, gp C-EDTA 0.04 M	61
C4	$4.8 \times 10^{12}$	EAC1gp†	C2hu, gp C-EDTA 0.04 M	95
C2	$5.0 \times 10^{10}$	EAC1gp4hu	gp C-EDTA 0.04 M	90
C3	$1.4 \times 10^{11}$	EAC1gp4hu	C2, 5, 6, 7hu C8, 9, gp	11

Normal human serum was diluted with gelatin-veronal buffer containing 0.0015 M Ca<sup>2+</sup> and 0.0005 M Mg<sup>2+</sup> to provide the above effective molecules of individual components. One ml was incubated with 270 µg of the variant antigen, or buffer alone, for 1 h at 37 °C. The residual activities of individual components were then assayed using cellular intermediates and functionally pure components from Cordis Laboratories, Miami. The cellular intermediates were used at a concentration of  $1 \times 10^8$  cells per ml.

\*hu, Human.

†gp, guinea pig.

and in animals suffering from trypanosomiasis. Since recent experiments have shown that complement is essential in antibody-mediated destruction of trypanosomes<sup>13</sup>, the hypocomplementaemic state resulting from the activation would favour the survival of the parasites.

Papamichail *et al.*<sup>14</sup> and others have shown that complement plays a part in antibody production to thymus-dependent antigens. Whether or not trypanosome antigens are thymus-dependent is uncertain. But, such low complement levels are likely to affect the antibody response of the host to trypanosome antigens.

Finally, evidence of increased vascular permeability in the form of perivascular oedema and swelling and degeneration of vessel walls, is a consistent pathological finding in animals infected with *Tr. brucei*<sup>15,16</sup> and *Tr. congolense*<sup>17</sup>. While these changes might result from antigen-antibody reactions, our observation suggesting interaction between variant antigen and complement, producing a vascular permeability factor, should also be considered as a mechanism in the pathogenesis of these and other lesions which occur in African trypanosomiasis<sup>18</sup>.

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*Note added in proof:* Activation of complement unidentified factors from *Tr. congolense* and *Tr. lewis* (Nielsen, K. & Sheppard, J. *Experientia* **33**, 769-770 (1977)).

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1. Hammerberg, B., Musoke, A. J., Huestead, S. T. & Williams, J. F. in *Pathophysiology of Parasitic Infections* (ed. Soulsby, E. J. L.) 233-240 (Academic, New York, 1976).
2. Machado, A. J., Gazzinelli, G., Pellegrino, J. & Dias Da Silva, W. *Expl Parasit.* **38**, 20-39 (1975).
3. Jarvinen, J. A. & Dalmasso, A. P. *Infection Immunity* **4**, 894-902 (1976).
4. Nagle, R. B. *et al.* *Am. J. trop. Med. Hyg.* **23**, 15-26 (1974).
5. Kobayashi, A. & Tizard, I. R. *Tropenmed. Parasit.* **27**, 411-417 (1976).
6. Lambert, P. H. & Houba, V. in *Progr. Immun.* **11**, 57-67 (1974).
7. Murray, M., Lambert, P. H. & Morrison, W. I. *Medicine Malad. Infect.* **12**, 638-641 (1975).
8. Lanham, S. M. *Nature* **218**, 1273-1274 (1968).
9. Rapp, H. J. & Borsoos, T. in *Molecular Basis of Complement Action* (Appleton-Century-Crofts, New York, 1970).
10. Cross, G. A. M. *Parasitology* **71**, 393-417 (1975).
11. Fine, D. P., Marney, S. R., Colley, D. G., Sergeant, J. S. & Des Prez, R. M. *J. Immunology* **109**, 807-809 (1972).
12. Greenwood, B. M. & Whittle, H. C. *Clin. exp. Immunol.* **24**, 133-138 (1976).
13. Diggs, C. *et al.* *J. Immunol.* **116**, 1005-1009 (1976).
14. Papamichail, M., Gutierrez, C., Embling, P., Holborrow, E. J. & Pepys, M. B. *Scand. J. Immunol.* **4**, 343-347 (1975).
15. Murray, M., Murray, P. K., Jennings, F. W., Fisher, E. W. & Urquhart, G. M. *Res. Vet. Sci.* **16**, 77-84 (1974).
16. Goodwin, L. G. *Trans. R. Soc. trop. Med. Hyg.* **82**-88 (1971).
17. Banks, K. L. *Infect. Immun.* (Submitted).
18. Murray, M. in *Progr. Immunol.* **11**, 5, 181-192 (1974).

## Induction of epidermal transglutaminase by hydrocortisone in chick embryonic skin

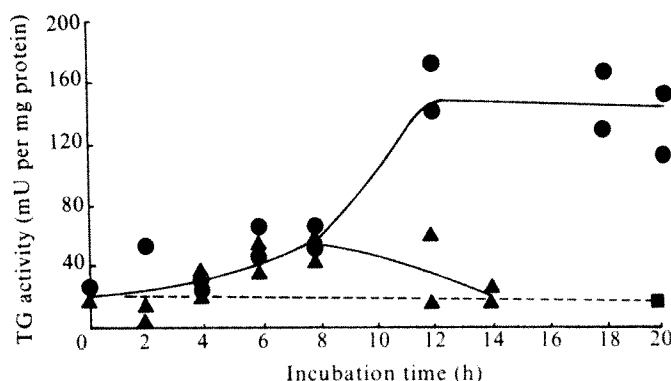
WE demonstrated previously that the epidermal cells of chick embryos are induced to differentiate by glucocorticoids<sup>1,2</sup>. Hydrocortisone ( $0.01 \mu\text{g ml}^{-1}$ ) added to a serum-free chemically-defined medium produced an intense keratinised layer over the uppermost cells<sup>3</sup> and many tonofilaments within the basal and intermediate cells<sup>4</sup> of the epidermis after 4 d in culture of 13-d-old embryonic chick tarsometatarsal skin. Within 12-16 h of hydrocortisone addition it is possible to show the synthesis of a urea-mercaptoethanol soluble, glycine-rich and metabolically stable epidermal structural protein<sup>5</sup> and the accumulation of a urea-mercaptoethanol insoluble epidermal protein(s)<sup>4</sup>. These changes of protein metabolism induced by hydrocortisone paralleled those seen during *in ovo* keratinisation of the epidermis<sup>4,5</sup>. The amino acid composition of the two proteins was very similar suggesting that the soluble protein is converted to the insoluble one in the process of epidermal keratinisation of chick embryonic skin. We show here that the levels of transglutaminase (TG), an enzyme that possibly mediates cross-linking of keratinous polypeptide chains by a  $\epsilon$ -( $\gamma$ -glutamyl) lysine bond<sup>6-8</sup>, increases both during *in ovo*

development of the epidermis and during hydrocortisone-induced *in vitro* keratinisation of chick embryonic cultured skin.

Table 1 shows that TG activity in the undifferentiated epidermis of 13-d-old embryonic chick skin was only 7 mU per mg protein, compared with 53 mU per mg protein in the heavily keratinised epidermis of 19-d-old embryonic chick skin. Furthermore, when 13-d-old embryonic chick skin was cultured for 4 d in a chemically-defined medium with or without hydrocortisone, TG activities of the steroid-treated epidermis were 52-107 mU per mg protein, whereas the activity in control epidermis was only 3-30 mU per mg protein. Figure 1 shows that after culture for 20 h the specific activities of TG in the treated epidermis were seven times those in control tissue. During the cultivation epidermal TG activities started to rise sharply after incubation for 8 h in the presence of the steroid. But, when actinomycin D was added at  $4 \mu\text{g ml}^{-1}$ , a concentration that blocks more than 99% of RNA synthesis, no increase of TG activity was observed, indicating that TG induction by hydrocortisone requires RNA synthesis. A similar effect was seen with cultured chick embryonic neural retina<sup>9</sup>; hydrocortisone added to the medium induced glutamine synthetase followed by *in vitro* differentiation of the tissue and actinomycin D abolished the enzyme induction. Further analyses are necessary to clarify whether hydrocortisone stimulates the synthesis of TG mRNA in our skin culture; however glucocorticoids were reported to induce the synthesis of specific enzymes through the increased synthesis of their mRNA for cultured rat hepatoma cells<sup>10</sup> and for rat liver<sup>11</sup> while many other kinds of cells, tissues and organs require the steroids for induction or maintenance of their differentiated function<sup>12</sup>.

It should be noted that hydrocortisone induced TG several hours before the induction of soluble epidermal structural protein and this earlier increase of the enzyme activity coincided exactly with that seen during *in ovo* development of the epidermis (data not shown). These findings suggest that the enzyme may be involved in a series of finely organised processes of epidermal cell differentiation. But more detailed studies are required to test the hypothesis that soluble structural proteins are cross-linked or polymerised by epidermal TG to insoluble high molecular ones composing tonofilaments to culminate in keratinisation of epidermal cells. One of the problems under study is whether

**Fig. 1** Changes of the specific activities of epidermal transglutaminase (TG) of 13-d-old chick embryonic skin during the cultures in the presence or absence of inducer and suppressor. Methods of skin cultivation and TG determination were essentially as described in Table 1. One of the pair-mate explants after preculture for 1 d was incubated with hydrocortisone (HC;  $0.01 \mu\text{g ml}^{-1}$ ) and the other with the steroid plus actinomycin D (Boehringer Mannheim;  $4 \mu\text{g ml}^{-1}$ ). ■, Epidermal TG activities of the skin cultured without HC; ●, epidermal TG activities of the skin cultured with HC; ▲, epidermal TG activities of the skin cultured with HC plus actinomycin D. Values of each point are the mean of duplicated determinations for a pool of five sheets of the epidermis.



**Table 1** Increase of epidermal transglutaminase activities (mU per mg protein) with keratinisation *in ovo* and *in vitro*

<i>In ovo</i>		<i>In vitro</i>	
Undifferentiated epidermis from 13-d-old embryos	Keratinised epidermis from 19-d-old embryos	Undifferentiated epidermis without hydrocortisone	Keratinised epidermis with hydrocortisone
		Expt 1	26 30
7 ± 1(5)*	53 ± 4(4)*		93 107
		Expt 2	9 3
			52 52

Pair-mate explants from right and left tarsometatarsal regions of 13-d-old chick embryos were cultured for 4 d in a chemically-defined medium, BGJb supplemented with ascorbate, by the Millipore filter-roller-tube method<sup>3</sup>. One of the pair-mate explants was cultured in the medium containing hydrocortisone sodium hemisuccinate (Upjohn) 0.01 µg ml<sup>-1</sup>, and the other was cultured in medium without the steroid. The epidermis of cultured explants and that from the same part of chick embryos in the normal course to hatching were each obtained by incubating the skin at 37 °C for 10–40 min in Eagle's minimum essential medium supplemented with 10% calf serum and 900 PU per ml Dispase I (a bacterial neutral protease, Godo Shusei). Crude enzyme preparation from the epidermis was prepared by a modification of the method of Ogawa and Goldsmith<sup>6</sup>. The epidermis was homogenised in 10 mM Tris-acetate (pH 7.5)–1 mM EDTA by the use of a glass homogeniser with five strokes at intermediate speed. This and all further operations were at 0–4 °C. The homogenates were centrifuged for 30 min at 12,000g at 4 °C. The sediments were extracted two additional times in the same manner and the supernatants were combined. The supernatant fluid taken in a cellulose tubing (Visking) was concentrated with sucrose. The concentrated supernatant was dialysed against a buffer of 5 mM Tris-acetate (pH 7.5) with 1 mM EDTA. Transglutaminase activity was assayed for the dialysed supernatant as an enzyme preparation by measuring the incorporation of [1,4-<sup>14</sup>C]putrescine (Radiochemical Centre, Amersham; 60 mCi mmol<sup>-1</sup>) into α-casein (according to Hammarsten; Merck) after the method of Goldsmith and Martin<sup>13</sup>. The assay mixture contained 0.11 M glycine-NaOH (pH 10.0) with 0.56 mM EDTA, 10 mM CaCl<sub>2</sub>, 5 mM dithiothreitol, 9 µM labelled putrescine and 0.15% casein. Aliquots (0.5 ml) of the assay mixture were added to 100 µl of each enzyme preparation. The mixtures were incubated for 30 min at 37 °C. The reaction was stopped by the addition of 0.6 ml of 10% trichloroacetic acid. The precipitate was collected on Whatman GF/C filters and the precipitated radioactivity was measured in toluene containing 0.4% 2,5-diphenyloxazole (Dotite) and 0.01% 1,4-bis(2-(5-phenyloxazolyl))-benzene (Dotite) with a Aloka Liquid Scintillation Spectrometer. An enzyme unit is defined as the amount of enzyme that catalyses the incorporation of 1 nmol of putrescine into casein in 30 min. Specific activity is defined as enzyme units per mg protein. Protein was measured by the technique of Lowry *et al.*<sup>14</sup> with bovine serum albumin as a standard. Values are averages of duplicated determinations for a pool of five sheets of the epidermis.

\*Mean ± standard error. Figures in parentheses represent the number of separate experiments.

there are much more ε- (γ-glutamyl) lysine cross-links in the insoluble protein than in the soluble protein of the chick embryonic epidermis.

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- Endo, H. *et al.* in *Biochemistry of Cutaneous Epidermal Differentiation* (eds Seiji, M. & Bernstein, A.) 359–378 (University of Tokyo Press, Tokyo, 1977).
- Sengel, P. in *Morphogenesis of Skin* 133–136 (Cambridge University Press, Cambridge, 1976).
- Sugimoto, M. & Endo, H. *J. Embryol. exp. Morph.* **25**, 365–376 (1971).
- Sugimoto, M., Tajima, K., Kojima, A. & Endo, H. *Dev. Biol.* **39**, 259–307 (1974).
- Kojima, A., Sugimoto, M. & Endo, H. *Dev. Biol.* **48**, 173–183 (1976).
- Ogawa, H. & Goldsmith, L. A. *J. biol. Chem.* **251**, 7281–7288 (1976).
- Buxman, M. M. & Wuepper, K. D. *J. invest. Dermatol.* **65**, 107–112 (1975).
- Abernethy, J. L., Hill, R. L. & Goldsmith, L. A. *J. biol. Chem.* **252**, 1837–1839 (1977).
- Moscona, A. A., Moscona, M. H. & Saenz, N. *Proc. natn. Acad. Sci. U.S.A.* **61**, 160–167 (1968).
- Steinberg, R. A., Levinson, B. B. & Tomkins, G. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2007–2011 (1975).
- Schutz, G., Killewich, L., Chen, G. & Feigelson, P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1017–1020 (1975).
- Sugimoto, M., Kojima, A. & Endo, H. *Dev. Growth Different.* **18**, 319–327 (1976).
- Goldsmith, L. A. & Martin, C. M. *J. invest. Dermatol.* **64**, 316–321 (1975).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).

## Ionophore-mediated calcium influx effects on the post-synaptic muscle fibre membrane

THE physiological chemotransmitter acetylcholine and its structural analogues such as carbamylcholine are capable of producing a sustained blockade of neuromuscular transmission when applied to the neuromuscular junction for prolonged periods. This neuromuscular blockade is not due to a depolarisation of the postsynaptic muscle fibre membrane but rather is generally ascribed to the inactivation, or 'desensitisation' of cholinergic receptor molecules in the postsynaptic membrane<sup>1,2</sup>. Various kinetic models involving agonist, and receptor

molecules postulated to exist in different conformational states, have been proposed to account for the time course of the desensitisation process<sup>3,4</sup>. These models do not incorporate the acceleration of desensitisation by calcium ions<sup>4,5</sup> and they do not predict correctly the observed effects of certain cholinergic antagonists on desensitisation. Consequently, alternative models for desensitisation have been proposed. In one of them calcium ions accumulate at the interior surface of the post-synaptic membrane and bind to the cholinergic receptors, thereby causing desensitisation by producing or sustaining some inactive receptor conformation<sup>6</sup>. We tested the model for desensitisation which involves internal calcium binding. This was accomplished by facilitating calcium ion flux across the post-synaptic membrane using the divalent cation ionophore A23187, while simultaneously producing a rapid desensitisation with iontophoretic application of carbamylcholine. Our results provide further evidence that calcium ions are a significant factor in the molecular mechanism of desensitisation.

A23187 is a carboxylic ionophore with high affinity for divalent cations and little or no affinity for univalent cations<sup>7</sup>. It is capable of transporting calcium ions across otherwise highly impermeable artificial and biological membranes by an essentially electroneutral process<sup>8</sup>. As a probe in research on contractile systems, A23187 has been used to release stored calcium from isolated sarcoplasmic reticulum vesicles<sup>9,10</sup>. A23187 has also been reported to increase the twitch tension of barnacle muscle fibres without altering the electrical properties of the fibre membranes<sup>11</sup>.

Using established single fibre stimulation and recording techniques, we investigated the effects of a wide range of A23187 concentrations on the electrical behaviour of frog (*Rana pipiens*) cutaneous pectoris or sartorius muscles employed in our desensitisation experiments. Resting and action potentials were recorded at junctional and extra-junctional sites and in addition, miniature end-plate potentials (m.e.p.s) were recorded at the junctions.

Because A23187 is only slightly soluble in aqueous media such as frog Ringer solution, a stock solution was prepared in 100% ethanol and appropriate quantities were added to the Ringer solution. Control experiments showed that ethanol, at the highest concentrations involved (0.5%), had an insignificant effect on the resting potentials, action potentials and m.e.p. frequencies recorded from muscle fibres. A small increase in



**Table 1** Effects of bath-applied A23187 on miniature end-plate potential frequency

Expt	A23187 Concentration ( $\mu\text{M}$ )	Exposure time (min)	M.e.p. frequency (Hz)	No. of fibres
1	0 (control)	Continuous	$0.30 \pm 0.22^*$	10
	0.28		$0.57 \pm 0.13$	12
2	0 (control)	Continuous	$1.1 \pm 0.7$	6
	0.56		$1.4 \pm 1.3$	6
3	0 (control)	10	$2.0 \pm 0.3$	10
	3.0		$4.8 \pm 1.2$	11
4	0 (control)	5	$1.3 \pm 0.6$	10
	7.5		$6.0 \pm 3.9$	14

Control solution was HEPES buffered Ringer solution (composition: 112.4 mM  $\text{Na}^+$ , 2.5 mM  $\text{K}^+$ , 1.8 mM  $\text{Ca}^{2+}$ , 117.1 mM  $\text{Cl}^-$ , 3.0 mM HEPES buffer; pH, 7.4). A23187 solutions contained 0.02, 0.04, 0.2 and 0.5% ethanol respectively.

\*Mean  $\pm$  s.d.

m.e.p. frequency was obtained when the Ringer solution contained 1% ethanol; this agrees with published data on the effects of alcohol on neuromuscular systems<sup>12,13</sup>. In our experiments involving the production of desensitisation the alcohol concentration never exceeded 0.04%. Control experiments showed that at this concentration ethanol had no effect on desensitisation.

Two different methods were used to apply the ionophore to the muscle fibres in both electrical behaviour studies and desensitisation studies. In the first method A23187 stock solution was added to HEPES-buffered Ringer solution (see Table 1 for composition) forming a suspension of the ionophore. After making recordings from muscle fibres bathed in ionophore-free Ringer solution, the low concentration A23187 solutions were continuously bath-applied during which time a second group of recordings was obtained. With solutions containing high concentrations of A23187 (3–15  $\mu\text{M}$ ) application to the muscle was limited to a few minutes following which the bath was changed to ionophore free Ringer solution and thereafter additional recordings were made. This procedure was used to avoid production of 'retraction clots' and other mechanical disturbances presumably caused by an excessive ionophore-promoted increase in  $\text{Ca}^{2+}$  influx which could lead to activation of the contractile system.

Many fibres exposed to A23187 for 5–10 min at concentrations above 8  $\mu\text{M}$  contracted irreversibly (formed retraction clots) and others twitched spontaneously for several minutes after the ionophore was removed from the bath. Retraction clots were also produced in fibres perfused with 6  $\mu\text{M}$  to 8  $\mu\text{M}$  A23187 solutions.

In the second method of applying the A23187, a quantity of the ionophore stock solution was added to calcium-free Ringer solution. A small region of a muscle immersed in ordinary Ringer solution was then microprefused with the Ca-free A23187 solution for 1–2 min. Recordings were made at the perfusion sites after allowing the ordinary Ringer solution to diffuse back into the ionophore treated region. This perfusion method was used in order to avoid mechanical artefacts (see above).

From bath application experiments we found that A23187, at concentrations below 1.6  $\mu\text{M}$ , had no effect on the muscle fibre resting and action potentials. With cells loaded at higher ionophore concentrations (up to 15  $\mu\text{M}$ ) the resting potentials were slightly diminished and the action potentials showed reduced rates of rise and fall. Statistical analysis, however, indicated that these differences are of doubtful significance, in contrast with the marked effects observed in experiments utilising the divalent cation ionophore X537A (ref. 14).

All of the A23187 solutions used in our experiments caused some increase in m.e.p. frequency; typical values are given in Table 1. The small increase from 1.1 to 1.4 s<sup>-1</sup> at 0.56  $\mu\text{M}$  A23187 is not statistically significant, but the differences do become

significant at the higher ionophore concentrations. Often, with A23187 applied at high concentrations (bath or perfusion), prolonged high frequency bursts (showers) of m.e.ps were observed, followed by decreased frequencies below control levels.

The above findings indicated that A23187 functioned as an electroneutral calcium ionophore in the skeletal muscle preparations, since calcium ion influx triggers both quantal release of acetylcholine from nerve terminals<sup>15</sup> and activates the contractile mechanism in muscle fibres<sup>16</sup>. Further support for this conclusion was obtained when perfusion of 6  $\mu\text{M}$  A23187 solution ( $\text{Ca}^{2+}$ -free) failed to produce either retraction clots or increased m.e.p. frequencies in fibres bathed in Ringer solution in which the  $\text{Ca}^{2+}$  had been replaced with  $\text{Mg}^{2+}$ .

Desensitisation experiments were performed using constant current iontophoretic pulses of carbamylcholine<sup>17</sup>. The applied current pulses flowing through the iontophoretic micropipette and the corresponding evoked membrane potential changes were monitored continuously. Endplate regions of high sensitivity to the agonist were first located using single 5–10 ms duration iontophoretic pulses giving approximately 1 mV depolarisation responses, thus avoiding any preliminary desensitisation effects<sup>18</sup>. Trains of iontophoretic pulses ranging from 0.4 s duration at 1.0 Hz to 1.0 s at 0.5 Hz were then generated.

During delivery of the first few iontophoretic pulses, the evoked membrane potential changes produced by the applied carbamylcholine generally increased in amplitude over the

**Table 2** Desensitisation half-times,  $\tau_{1/2}$ , for frog skeletal muscle exposed to A23187

Fibre	Resting potential (mV)	$\Delta V_{\text{max}}$ (mV)§	$\Delta V_f$ (mV)¶	$\tau_{1/2}$ (s)
1, Control*	88	14.5	8.8	11.5
1, Perfused†	83	15.0	9.2	8.0
2, Control	85	9.5	5.6	16.0
2, Perfused†	87	10.6	2.0	12.0
3, Control	89	0.6	0.4	10.4
3, Perfused†	85	0.6	0.4	4.2
4, Control	84	2.9	2.9	—¶
4, Perfused†	88	3.5	2.5	15.0
5, Control	92	6.3	3.5	12.0
5, Perfused†	92	4.2	1.0	7.0
6, Control	85	23.8	12.6	13.7
6, Bath‡	90	20.0	13.7	8.2
7, Control	85	36.5	32.5	12.7
7, Bath‡	87	16.0	10.6	7.9
8, Control	87	9.0	6.0	19.0
8, Bath‡	88	10.0	4.0	10.5
9, Control	83	7.5	6.0	25.0
9, Bath‡	86	11.2	5.0	15.0
10, Control	83	10.0	4.8	12.0
10, Bath‡	85	11.2	8.5	8.5
11, Control	85	11.2	8.0	11.5
11, Bath‡	88	12.0	6.2	7.5
Means				
Control	86	12.0	8.3	14.4
Experimental	87	10.4	5.7	8.9

\*Ringer solution contains 1.8 mM  $\text{Ca}^{2+}$  all fibres below bathed in Ringer solution containing 3.6 mM  $\text{Ca}^{2+}$ .

†After the control period, the fibre was perfused with 2.4  $\mu\text{M}$  A23187 solution ( $\text{Ca}^{2+}$ -free ethanol–0.16%) for 1 to 2 min; recordings were then repeated 10 to 15 min after perfusion.

‡After the control period, 0.56  $\mu\text{M}$  A23187 (0.04% ethanol) was bath applied to the fibre and recordings were repeated in the presence of the ionophore after waiting 30 to 60 min.

§Maximum depolarisation pulse amplitude (see Fig. 1).

¶Final (steady-state) depolarisation pulse amplitude (see Fig. 1).

¶No desensitisation.

first few pulses to a maximum,  $\Delta V_{\max}$  then declined in amplitude to a final steady response  $\Delta V_f$  as illustrated in Fig. 1. The phenomenon of increasing  $\Delta V$  amplitudes which occurs with the first few iontophoretic pulses is not an artefact but represents a physiological potentiation phenomenon<sup>19,20</sup>. The decline in evoked potential amplitude which, occurred after the maximum response was obtained, indicated that desensitisation was in progress. The time  $\tau_1$  for these depolarisations to decline from  $\Delta V_{\max}$  to  $\frac{1}{2}(\Delta V_{\max} + \Delta V_f)$  was taken as a measure of the desensitisation rate. Comparisons of control and ionophore treated fibres were made only between recordings taken at the same postjunctional location on a given fibre because under control conditions there has been found to be a large variation in desensitisation rates amongst endplates from the same muscle<sup>18</sup>.

The results of these desensitisation experiments, presented in Table 2, demonstrated that very low concentrations of A23187 accelerated the desensitisation rate by 30% to 60%. For example, the desensitisation half time for fibre 1, which was 11.5 s in the control, decreased to 8.0 s after perfusion with 2.4  $\mu\text{M}$  A23187 solution. Apparently A23187 did not alter the sensitivity of the fibres to carbamylcholine, because following its application inconsequential random changes in the value of  $\Delta V_{\max}$  occurred. Direct measurement of endplate sensitivities, determined as membrane response in  $\text{mV nC}^{-1}$  of applied iontophoretic drug, confirmed this conclusion. Thus A23187 did not promote desensitisation by direct antagonistic interaction with the cholinergic receptors.

From the data we and others have obtained showing that A23187 carries calcium ions across excitable membranes, it could be concluded that the substantial acceleration of desensitisation by the ionophore was due to its action in increasing calcium ion influx both in the resting and the carbamylcholine activated postsynaptic membrane. We wondered what increase in intracellular calcium concentration would be produced by the action of A23187 alone. The concentrations of A23187 used in the desensitisation experiments were well below those at which retraction clots or changes in the electrical characteristics of the excitable membrane were produced. Thus if the minimal concentration of  $\text{Ca}^{2+}$  required to activate the contractile mechanism<sup>21</sup> is  $10^{-6}$  M then one might conclude that the  $[\text{Ca}^{2+}]_i$  in these ionophore loaded fibres was less than  $10^{-6}$  M before development of desensitisation by application of carbamylcholine.

Although the ionophore produced increase in  $[\text{Ca}^{2+}]_i$  seems too be small, its effect become evident during application of

desensitising iontophoretic pulses of carbamylcholine which further increase  $\text{Ca}^{2+}$  influx across the postjunctional membrane. We attempted to calculate the increase in  $[\text{Ca}^{2+}]_i$  resulting from the action of A23187 alone but the value obtained was clearly too high and we attributed this apparently erroneous result to the many questionable assumptions involved in the calculation.

In the above speculations it is tacitly assumed that the influxing  $\text{Ca}^{2+}$  ions are free to distribute themselves in the adjacent intracellular fluid. As suggested earlier<sup>6</sup>, however, the possibility exists that during production of receptor desensitisation influxing  $\text{Ca}^{2+}$  ions become bound to anionic sites on the interior surface of the postjunctional membrane and also accumulate in the intracellular fluid immediately adjacent to this membrane. In this model a relatively small amount of influxing  $\text{Ca}^{2+}$  could produce a substantial localised increase in calcium concentration. Such a localised increase in calcium would not necessarily cause a retraction clot because, as can be seen in electron micrographs, the intracellular region immediately adjacent to the postjunctional membrane does not contain contractile elements and in addition, some of the intracellular calcium would not be free.

The effect of the increase in  $[\text{Ca}^{2+}]_i$  produced by A23187 becomes evident during application of desensitising iontophoretic pulses of carbamylcholine to the postjunctional membrane which further increase  $\text{Ca}^{2+}$  influx. We suppose that  $\text{Ca}^{2+}$  ions exert some positive cooperative action in stabilising an inactive receptor state, or a closed ion channel conformation. Binding studies with purified acetylcholine receptor (AChR) have shown that ACh receptor has a fairly high affinity for  $\text{Ca}^{2+}$  and that  $\text{Ca}^{2+}$  influences the affinity of ACh receptor for cholinergic agonists<sup>22-24</sup>. If we assume that the agonist-receptor complex goes spontaneously through a series of active and then inactive states, a small localised increase in  $\text{Ca}^{2+}$  might maintain an ever increasing number of receptors in a complex but inactive state.

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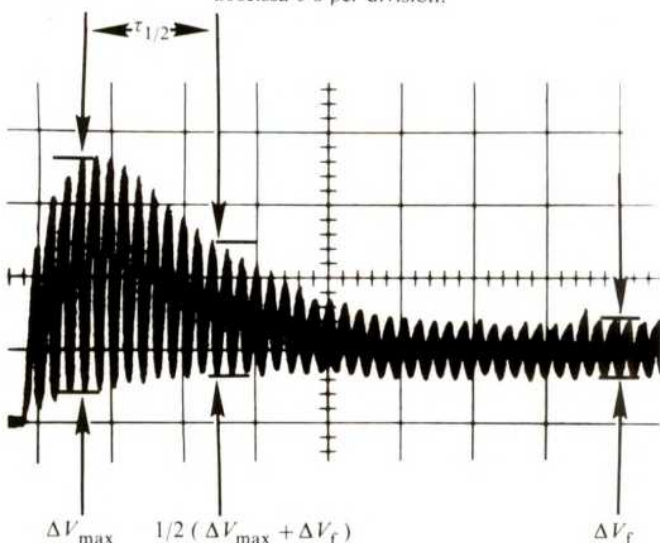
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- Thesleff, S. *Acta physiol. scand.* **34**, 218-231 (1955).
- Katz, B. & Thesleff, S. *J. Physiol., Lond.* **138**, 63-80 (1957).
- Rang, H. P. & Ritter, J. M. *Molec. Pharmac.* **6**, 357-382 (1970).
- Manthey, A. A. *J. gen. Physiol.* **49**, 963-975 (1966).
- Magazanik, L. G. & Vyskocil, F. J. *Physiol., Lond.* **210**, 507-518 (1970).
- Nastuk, W. L. & Parsons, R. L. *J. gen. Physiol.* **56**, 218-249 (1970).
- Reed, P. W. & Lardy, H. A. in *The Role of Membranes in Metabolic Regulation* (eds Mehlman, M. A. & Hanson, R. W.) 111-131 (Academic Press, New York, 1972).
- McLaughlin, S. & Eisenberg, M. A. *Rev. Biophys. Bioengng* **4**, 335-366 (1975).
- Caswell, A. H. & Pressman, B. C. *Biochem. biophys. Res. Commun.* **49**, 292-298 (1972).
- Scarpa, A., Baldassare, J. & Inesi, G. *J. gen. Physiol.* **60**, 735-749 (1972).
- Hainut, K. & Desmedt, J. E. *Nature* **252**, 407-408 (1974).
- Inoue, F. & Frank, G. B. *Br. J. Pharmac. Chemother.* **30**, 186-193 (1967).
- Okada, K. *Jap. J. Physiol.* **17**, 245-261 (1967).
- Devore, D. I. & Nastuk, W. L. *Nature* **253**, 644-646 (1975).
- Miledi, R. *Proc. R. Soc. B183*, 421-425 (1973).
- Huxley, A. F. *J. Physiol., Lond.* **243**, 1-43 (1974).
- Nastuk, W. L. *Fedn Proc.* **12**, 102 (1953).
- Wolfson, C. H. & Nastuk, W. L. *Fedn Proc.* **34**, 404 (1975).
- Hartzell, H. C., Kuffler, S. W. & Yoshikami, D. *J. Physiol., Lond.* **251**, 427-463 (1975).
- Wolfson, C. H. thesis, Columbia Univ. (in preparation).
- Ebashi, S., Endo, M. & Ohtsuki, I. *Q. Rev. Biophys.* **2**, 351-384 (1969).
- Martinez-Carrion, M. & Raftery, M. A. *Biochem. biophys. Res. Commun.* **55**, 1156-1164 (1973).
- Cohen, J. B., Weber, M. & Changeux, J.-P. *Molec. Pharmac.* **10**, 904-932 (1974).
- Chang, H. W. & Neumann, E. *Proc. natn. Acad. Sci. U.S.A.*, **73**, 3364-3368 (1976).

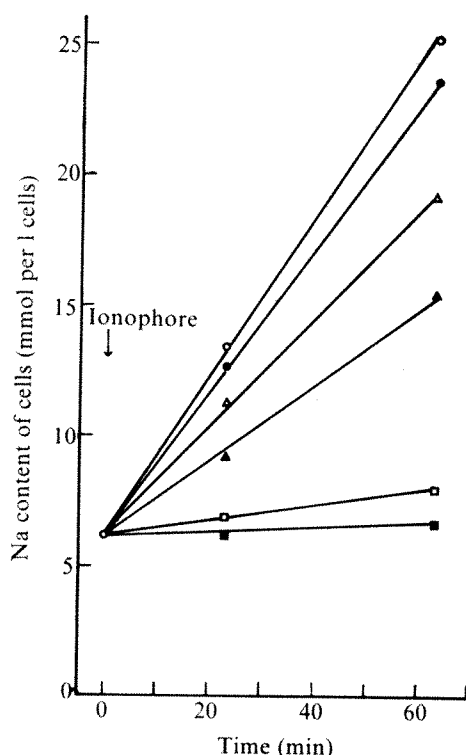
Fig. 1 Typical membrane depolarisations,  $\Delta V$ , produced during iontophoretic application of a 1 Hz train of 0.7 s constant current carbamylcholine pulses to the muscle postjunctional membrane. Note the early facilitation followed by later developing desensitisation. Calibrations: ordinate 0.5 mV per division, abscissa 5 s per division.





## Does ionophore A23187 mediate Na transport in the absence of divalent cations?

THE divalent cation-selective ionophore A23187 is known to form complexes with monovalent cations<sup>1</sup>, but it is not clear whether such complexes actually translocate across biological membranes. In the absence of Ca and Mg, the ionophore can transport K across the mitochondrial membrane in a manner reminiscent of the nigericin class of ionophores<sup>2</sup>. From spectrofluorometric and bulk organic phase partition data<sup>3</sup>, Pfeiffer and Lardy concluded that Na binds to the ionophore better than K ( $\text{Li} \gg \text{Na} > \text{K} \approx \text{O}$ ) and it is surprising that no observations of this Na form of the ionophore crossing biological membranes have been reported. We have used a recently developed method<sup>3</sup> designed to study the effect of intracellular Mg on the various ion fluxes mediated by the Na pump, and we report here that intact human red cells incubated in buffered isotonic NaCl media containing EDTA, no added divalent cations and the ionophore



**Fig. 1** Effect of the ionophore A23187 on the uptake of sodium by intact red cells at various Mg concentrations. Red cells from 2-d-old bank blood were washed in Tris-buffered isotonic saline and resuspended at a haematocrit of 10% in a medium initially containing NaCl 150 mM, Tris-HCl 10 mM (pH 7.7 at 37 °C), Tris-EGTA 0.01 mM, Tris-EDTA 2 mM and ouabain  $10^{-4}$  M. The suspension was divided into six equal parts and  $\text{MgCl}_2$  was added from a concentrated stock solution (0.8 M) to give the initial total concentrations reported here ( $[\text{Mg}]_0^{t=0}$ ). Values of  $[\text{Mg}]_0^{t=0}$  were (mM):  $\circ$ , 0;  $\bullet$ , 0.5;  $\triangle$ , 1.0;  $\blacktriangle$ , 1.5;  $\square$ , 2.1;  $\blacksquare$ , Control (no ionophore). At  $t = 0$ , 5  $\mu\text{l}$  of absolute ethanol containing 1 mg  $\text{ml}^{-1}$  of ionophore A23187 were added (arrow) per ml of suspension during vigorous magnetic stirring (at 37 °C). Samples of 1 ml were taken at the indicated times. The cells were centrifuged and washed three times with 40 volumes of ice-cold Tris-buffered choline chloride solution. Na and K were determined by flame photometry. Concentrations are expressed in mmol per 1 original cell volume. The initial concentration of K in the cells was  $89.8 \pm 1.4$  and the final concentration was between 87.5 and 88.6 in the controls and high-Mg conditions and between 81.6 and 84.5 at the lowest Mg concentrations.

**Table 1** Effect of A23187 on unidirectional fluxes of  $^{24}\text{Na}$  in the presence or absence of Mg in low-Na media

Medium	Ionophore A23187	$[\text{Mg}^{2+}]_i$ (M)	Na fluxes (mmol per 1 cells per h)	
			Influx	Efflux
K	—	$\sim 3.2 \times 10^{-4}$	$0.210 \pm 0.014$	$0.836 \pm 0.009$
Choline	—	$\sim 3.2 \times 10^{-4}$	$0.137 \pm 0.008$	$1.377 \pm 0.009$
K	+	$3.2 \times 10^{-4}$	$0.198 \pm 0.008$	$0.747 \pm 0.013$
Choline	+	$3.2 \times 10^{-4}$	$0.147 \pm 0.006$	$1.216 \pm 0.017$
K	+	$\sim 10^{-7}$	$0.290 \pm 0.006$	$1.330 \pm 0.047$
Choline	+	$\sim 10^{-7}$	$0.292 \pm 0.012$	$1.495 \pm 0.021$

Fresh, washed human red cells were incubated with or without  $^{24}\text{Na}$  for 4 h at 37 °C in a Tris-buffered medium containing NaCl 75 mM and KCl 75 mM. The cells were then washed and suspended at a haematocrit of about 10% in a medium containing NaCl 5 mM, Tris-Cl 10 mM, Tris-EGTA 0.01 mM, Tris-EDTA 2 mM, ouabain  $10^{-4}$  M and 145 mM of either KCl or choline chloride.  $\text{MgCl}_2$  2.1 mM was present in groups three and four of the table giving the final concentrations of internal  $\text{Mg}^{2+}$  as indicated.  $^{24}\text{Na}$  was added to the suspension of tracer-free cells to measure Na influx. Na fluxes were calculated by multiplying the internal (expressed per 1 of cell water for efflux) and external (for influx) Na concentrations by the respective rate constants of tracer movement (the influx rate constant was corrected for backflux).

A23187, gain Na and swell. This effect was not accompanied by any change in the K permeability and could be prevented or reversed by adding Mg or Ca to the medium. Thus, in certain conditions A23187 may indeed mediate Na transport.

The effect of the ionophore A23187 on the bulk and tracer ( $^{24}\text{Na}$ ) fluxes of Na was investigated in intact red cells suspended in 'Ca-free' media containing ouabain, in order to avoid both Ca-induced changes in K permeability<sup>4,5</sup> and variations in Na-pump rate that occur at different internal Mg concentrations (to be reported elsewhere; see also ref. 6). Figure 1 shows the effect of the ionophore on the Na content of cells suspended in isotonic Na media containing increasing Mg concentrations. The nominally Mg-free cells ( $[\text{Mg}]_i \approx 10^{-7}$  M) gained about 18 mmol of Na per 1 of cells in 1 h, a value about 10 times the Na gain in ionophore-free cells or in cells containing ionophore and normal Mg. The net Na influx is plotted as a function of  $[\text{Mg}^{2+}]_i$  in Fig. 2, showing half-maximum inhibition in the micromolar range. The increase in Na flux was fully reversed by physiological concentrations of internal Mg. Ca was also found to inhibit the Na influx in the presence of the ionophore but this effect was masked to some extent by a separate action of Ca on the Na influx. Ca tended to increase the Na influx compared with Ca-free controls even in the presence of high concentrations of Mg, and made it difficult to assess the precise extent to which Ca inhibited the ionophore-mediated influx.

In order to avoid large changes in ion composition and cell volume, the effect of the ionophore on the unidirectional ( $^{24}\text{Na}$ ) tracer fluxes was investigated in media containing 5 mM Na with either K or choline as the main extracellular cation. The results of one such experiment are reported in Table 1. Here again it can be seen that the ionophore increases the unidirectional Na fluxes only in the absence of Mg. At the same time, however, the K content of the ionophore-treated cells was the same as that of untreated controls in a similar medium. Na efflux is increased relatively more when K is the main external cation, while the effect on the influx of Na is relatively larger in the choline medium. This seems to result from an apparent effect of external K on the mechanism which is responsible for the asymmetry between Na efflux and Na influx in the presence of ouabain<sup>7</sup> and which had been variously attributed in the past to a second Na-pump<sup>8</sup>, to counter-transport of Na

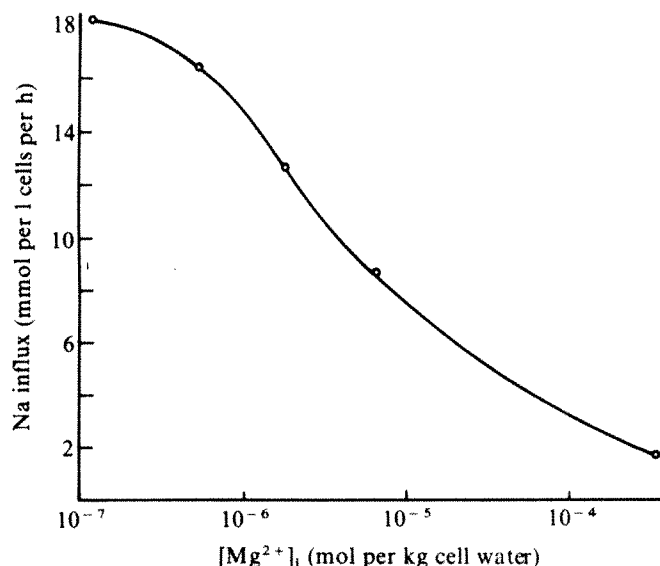


Fig. 2 Net gain of sodium as a function of the intracellular concentration of free Mg in intact red cells in the presence of the ionophore A23187. The Na uptake of Fig. 1 is plotted as a function of the  $Mg^{2+}$  content of the cells calculated from the ionophore-induced equilibrium distribution of Mg as reported previously<sup>3</sup>.

with choline or  $Mg^{2+}$  or to co-transport of Na with  $K^{10,11}$ . The present results rule out symmetric carrier-mediated transport involving choline, K or Mg, as Na efflux is larger than Na influx in all conditions, whether in the presence or absence of choline, K, or Mg gradients. The persistence of the asymmetric Na fluxes even in 'Mg-free' cells also argues against ATP-fuelled transport associated with a Mg-dependent ATPase<sup>12</sup>.

In order to discriminate between a possible indirect

effect of the ionophore and ionophore-mediated Na flux, we measured net Na influx in cells that had been treated with the ionophore in the presence and absence of Mg and from which the ionophore had been washed away<sup>5,13</sup>. This was done to see whether 'Mg-free' cells with little or no ionophore left could still exhibit the increased Na permeability. The results (Table 2) showed that the 'Mg-free' cells did not have an increased Na permeability unless the ionophore was restored during the final incubation. All the available evidence suggests either that the ionophore can act on the membrane to produce a reversible and selective increase in Na permeability at low  $Mg^{2+}$  or  $Ca^{2+}$  concentrations, an interesting possibility which cannot be ruled out, or, more likely, that the ionophore itself mediates Na transport when the concentration of competing divalent cations is very low.

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- Pfeiffer, D. R., Reed, P. W. & Lardy, H. A. *Biochemistry* **13**, 4007-4014 (1974).
- Pfeiffer, D. R. & Lardy, H. A. *Biochemistry* **15**, 935-943 (1976).
- Flatman, P. & Lew, V. L. *Nature* **267**, 360-362 (1977).
- Gardos, G. *Biochim. biophys. Acta* **30**, 653-654 (1958).
- Lew, V. L. & Ferreira, H. G. *Nature* **263**, 336-338 (1971).
- Bodemann, H. H. & Hoffman, J. F. *J. gen. Physiol.* **67**, 547-561 (1976).
- Beaugé, L. *Biochim. biophys. Acta* **401**, 95-108 (1975).
- Hoffman, J. F. & Kregenow, F. M. *Ann. N.Y. Acad. Sci.* **137**, 566 (1966).
- Beaugé, L. & Ortiz, O. J. *Membr. Biol.* **13**, 165-185 (1973).
- Sachs, J. R. *J. gen. Physiol.* **57**, 259-282 (1971).
- Lew, V. L. & Beaugé, L. in *Transport Across Biological Membranes* (eds Giebisch, G., Tosteson, D. C. & Ussing, H. H.) vol. 2 (Springer, in the press).
- Proverbio, F. M., Condrescu-Guidi & Whitembury, G. *Biochim. biophys. Acta* **394**, 281-292 (1975).
- Sarkadi, B., Szasz, L. & Gardos, G. *J. Membr. Biol.* **26**, 357-370 (1976).

Table 2 Effect of ionophore removal on Na influx in the presence and absence of internal Mg

Pretreatment of the cells	Ionophore A23187 during final incubation	Presence of Mg during final incubation	Na influx (mmol per 1 cells per h)
Controls	—	—	$2.76 \pm 0.104$
	—	+	$1.60 \pm 0.154$
	+	—	$25.79 \pm 0.461$
	+	+	$3.48 \pm 0.259$
Treated with ionophore in the absence of Mg	—	—	$3.43 \pm 0.459$
	—	+	$3.92 \pm 0.382$
	+	—	$26.99 \pm 0.590$
	+	+	$3.80 \pm 0.446$
Treated with ionophore in the presence of Mg	—	—	$1.97 \pm 0.236$
	—	+	$2.13 \pm 0.346$
	+	—	$28.84 \pm 0.708$
	+	+	$2.90 \pm 0.273$

Cells were pretreated with A23187 in the presence and absence of 2.1 mM  $MgCl_2$  in a Tris-buffered isotonic choline chloride medium containing 5 mM NaCl. A low sodium medium was used to prevent changes in the intracellular Na concentration during the preincubation. 5  $\mu$ l of a 1 mg ml<sup>-1</sup> solution of the ionophore in absolute ethanol was added for each ml of the suspension. The haematocrit was about 10% and the pre-incubation was performed at 37 °C for 30 min. The ionophore was absent from the control group, otherwise conditions were identical. The cells were subsequently washed five times in a similar ice-cold solution and resuspended in a medium containing NaCl 150 mM; Tris-HCl 10 mM (pH 7.7 at 37 °C), Tris-EGTA 0.01 mM, Tris-EDTA 2 mM and ouabain 10<sup>-4</sup> M.  $MgCl_2$ , when present, was at 2.1 mM. <sup>24</sup>Na was added to the final incubation medium in order to measure Na influx. When the ionophore was absent, the 'Mg-free' cells did not gain Mg in the Mg-containing media.

## Lowering of hypertension by central saralasin in the absence of plasma renin

A ROLE for the renin-angiotensin system in the development or maintenance of increased blood pressure of spontaneously hypertensive (SH) rats has not been substantiated. These animals are considered the best model for human essential hypertension. Although there have been reports of unusually high concentrations of renin in the plasma of young SH rats<sup>2</sup>, their pathophysiological relevance has been questioned<sup>3</sup> and, as we report here, high blood pressure is maintained even after nephrectomy. But angiotensin from the brain isorenin-angiotensin system (Iso-RAS) could be involved<sup>4,5</sup>. All the components of that system have been identified in brain tissue, including the enzyme iso-renin<sup>4-6</sup>, the substrate, angiotensinogen<sup>7</sup>, angiotensin I and converting enzyme<sup>6,8</sup> which hydrolyses angiotensin I to form the biologically active octapeptide angiotensin II. Angiotensin II is also present in brain tissue<sup>6</sup>. When it is injected intraventricularly into the brain angiotensin II produces a prolonged increase in blood pressure<sup>9</sup>. Therefore, if angiotensin II concentrations are increased in the brains of SH rats they could be involved in the hypertensive state independently of angiotensin levels from renal origin in plasma. Using the competitive antagonist Sar-1-Ala-8-angiotensin II (saralasin acetate, P113, Norwich Pharmacal) in SH and nephrectomised SH rats, we have confirmed this possibility.

Twenty-one SH stroke-prone (SH-SP) rats weighing 220-330 g and 10 age matched Wistar Kyoto control rats (WKY) were anaesthetised by ether, and a 28-gauge stainless steel cannula was implanted stereotactically into the right lateral ventricle. Rats were allowed 3-10 d for recovery and were



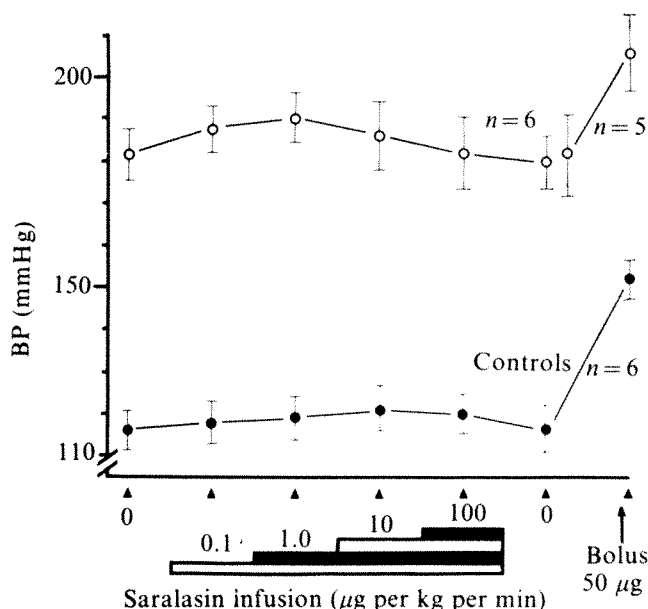
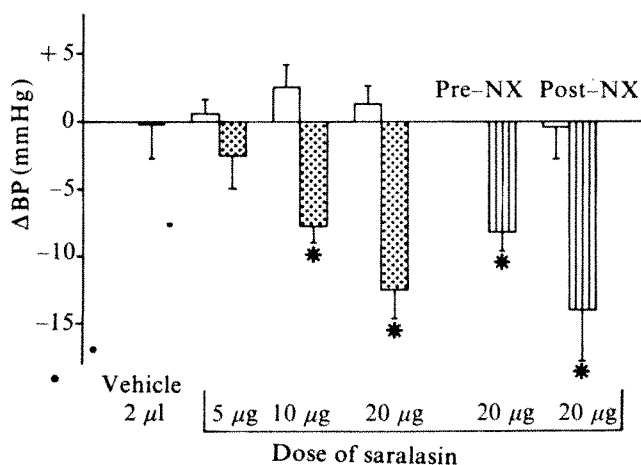
again anaesthetised with ether. A femoral vein catheter (polyethylene No. 10 tubing, Clay Adams, USA) and a femoral artery catheter (polyethylene No. 10 connected to No. 50 tubing) were inserted, ligatured and brought under the skin to exit through the scruff of the neck. A 1-ml blood sample was taken from the arterial catheter for plasma renin assay and simultaneously replaced by 1 ml isotonic saline through the venous catheter. Rats were left awake, freely moving in a wooden box during testing and recording. Blood pressure was recorded by a Statham transducer P 23 b and Hellige pre-amplifiers on to a Brush 220 polygraph. Testing began 90–120 min after blood pressure had become stable.

Ten SH-SP and six WKY male and female rats, all 4-months-old, were tested with intravenous and intraventricular doses of saralasin. Eleven SH-SP rats and four WKY rats (all females, 8-months-old) were nephrectomised and tested. Bilateral nephrectomy was carried out under ether anaesthesia and rats were tested 15–20 h later. Blood samples for plasma renin assays were also taken 15 h after nephrectomy.

To test the potency of the preparation each rat was tested initially with 100 ng angiotensin II intravenously and intraventricularly. After 1 h, saralasin was infused intravenously in cumulative doses of 0.1, 1.0, 10.0 and 100  $\mu\text{g}$  per kg per min for 15 min at each dose. Sixty min after the infusion test a bolus injection of 50  $\mu\text{g}$  saralasin was given intravenously. After 2–3 h recovery when blood pressure was stable, testing began with intraventricular injections of 5, 10 and 20  $\mu\text{g}$  saralasin in 0.5, 1.0 and 2.0- $\mu\text{l}$  volumes, respectively. The order of testing was randomised and there was full recovery of the original blood pressure between tests. Saralasin was dissolved in artificial cerebrospinal fluid and control injections of 2  $\mu\text{l}$  of this vehicle were also given.

The results are illustrated in Figs 1 and 2. The mean arterial blood pressure of SH-SP rats was  $181.3 \pm 9.2$  mmHg. Intraventricular saralasin lowered blood pressure in a dose-response relationship in every rat tested ( $n = 14$ ) (Fig. 1). In the 4-month-old rats 5  $\mu\text{g}$  saralasin produced a decrease of  $2.5 \pm 2.3$  mmHg. The 10- $\mu\text{g}$  dose lowered blood pressure by  $7.8 \pm 1.2$  mmHg and the 20- $\mu\text{g}$  dose by  $12.5 \pm 1.9$  mmHg ( $P < 0.01$  compared with controls by the *t*-test). A 20- $\mu\text{g}$  dose of saralasin lowered blood pressure in the 8-month-old rats by  $8.3 \pm 1.4$  mmHg ( $P < 0.01$  compared with controls). The decrease began in each case within 1 min of injection and reached a maximum by

**Fig. 1** Changes in mean arterial blood pressure ( $\Delta$  BP in mmHg) after injections of the angiotensin blocker saralasin into the cerebral ventricles of spontaneously hypertensive rats of the stroke-prone strain and into the cerebral ventricles of normotensive Wistar Kyoto control rats. Doses of intraventricular saralasin are indicated below. Pre-NX: rats tested before and in the same animals 16 h after bilateral nephrectomy (Post-NX). Dotted columns, 4-month-old ( $\bar{X} \pm \text{s.e.m.}$ ); open columns, age-matched controls; striped columns, 8-month-old.



**Fig. 2** The effect of intravenous saralasin infusions in cumulative doses on the mean arterial blood pressure (BP) of spontaneously hypertensive rats of the stroke-prone strain.

5–7 min. There was recovery back to the original blood pressure within 15–30 min. WKY rats matched for age showed no response to intraventricular saralasin (Fig. 1). When saralasin was given intravenously the two smallest doses caused increase of blood pressure in SH ( $n = 6$ ) and control rats ( $n = 6$ ) ( $P < 0.05$ ) (Fig. 2). Bolus injection of 50  $\mu\text{g}$  saralasin consistently produced an even more marked increase in blood pressure in both SH ( $23.4 \pm 2.5$  mmHg) and control rats ( $35.3 \pm 2.6$  mmHg) (Fig. 2).

To investigate whether circulating plasma angiotensin II of renal origin was involved in the central effect of saralasin we repeated the test in nephrectomised rats. In nephrectomised SH-SP rats the mean lowering of blood pressure by 20  $\mu\text{g}$  saralasin given intraventricularly was  $14.1 \pm 3.8$  mmHg. Intraventricular saralasin had no effect on blood pressure in nephrectomised control rats. Plasma renin activity in these rats was  $0.5 \pm 0.1$  ng angiotensin I  $\text{ml}^{-1} \text{h}^{-1}$ , which is close to detection limits of the assay and lower as compared with the control levels in SH rats of  $5.7 \pm 0.8$  ng angiotensin I  $\text{ml}^{-1} \text{h}^{-1}$ . Nephrectomy did not affect the blood pressure of control or experimental rats. The mean arterial pressure before nephrectomy was  $178.3 \pm 6.2$  mmHg and after nephrectomy it was  $178.0 \pm 6.3$  mmHg.

The response to angiotensin II in SH rats seemed to be greater than in WKY rats, as recently confirmed<sup>15</sup>.

These data implicate angiotensin in the central blood pressure regulation of SH rats. Although the effect was comparatively small, the consistency with which intraventricular saralasin lowered the blood pressure may reflect further factors which tend to maintain blood pressure in these rats. The effect is not readily obtained when blood pressure is lower<sup>16</sup>, nor was it obtained in normotensive control rats. The results also demonstrate that in the absence of peripheral plasma renin after nephrectomy, the blood pressure of SH rats is still increased.

The lowering of blood pressure by central saralasin in the absence of peripheral renin-angiotensin is evidence for a role of the brain iso-renin-angiotensin system in the maintenance of high blood pressure in SH-SP rats. Since its discovery<sup>1,5</sup>, several lines of evidence have suggested that the system has a physiological function. Electrophysiological evidence and recent biochemical binding studies have demonstrated angiotensin receptors in the brain<sup>10–13</sup>. Saralasin slowed the rate of firing of cells responsive to angiotensin<sup>10</sup>. This has been

interpreted to mean that the spontaneous rate was normally maintained at high rates by endogenous angiotensin. A reduction of blood pressure by central infusions or injections of angiotensin antagonists has been reported in other strains of SH rats (New Zealand and Okamoto strains)<sup>14-18</sup>, but negative findings have also been claimed<sup>19</sup>. Without nephrectomy these results were only circumstantial evidence for an action of extrarenal angiotensin. With the data reported here we conclude that extrarenal angiotensin acts on the brain receptors and contributes to the high blood pressure in SH-SP rats. In view of the growing support for the presence of locally formed brain angiotensin<sup>20</sup>, the brain iso-renin system is a likely extrarenal source.

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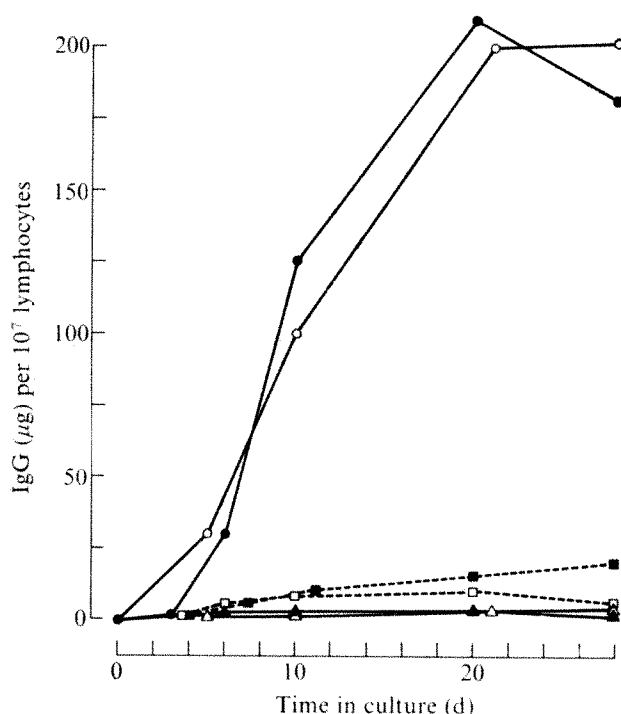
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- Okamoto, K. (ed.) *Spontaneous Hypertension: Its Pathogenesis and Complications* (Igaku Shoin Ltd, Tokyo, 1972).
- Sen, S., Smeby, R. R. & Bumpus, F. M. *Circ. Res.* **31**, 876-882 (1972).
- Kolitzky, S., Shook, P. & Rivera-Veley, J. in *Spontaneous Hypertension: Its Pathogenesis and Complications* (ed. Okamoto, K.) 199-202 (Igaku Shoin Ltd, Tokyo, 1972).
- Ganten, D. *et al. Science* **173**, 64-65 (1971).
- Fischer-Ferrario, C., Nahmod, V. E., Goldstein, D. J. & Finkelman, S. *J. exp. Med.* **133**, 353-361 (1971).
- Ganten, D., Hutchinson, J. S., Schelling, P., Ganten, U. & Fischer, H. *Clin. exp. Pharmacol. Physiol.* **2**, 103-126 (1976).
- Ganten, D. *et al. Am. J. Physiol.* **221**, 1733-1737 (1971).
- Yang, H.-Y. T. & Neff, N. H. *J. Neurochem.* **21**, 2443-2450 (1973).
- Severs, W. B. & Daniels-Severs, A. E. *Pharmacol. Rev.* **25**, 415-449 (1973).
- Phillips, M. I. & Felix, D. *Brain Res.* **110**, 531-540 (1976).
- Felix, D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **15**, 292-295 (1976).
- Nicoll, R. A. & Barker, J. L. *Nature new Biol.* **172**, 233-234 (1971).
- Bennet, J. P. & Snyder, S. H. *J. biol. Chem.* **251**, 7423-7430 (1976).
- Ganten, D., Hutchinson, J. S. & Schelling, P. *Clin. Sci. molec. Med.* **48**, 265s-268s (1975).
- Hoffman, W. E., Phillips, M. I. & Schmid, P. *Am. J. Physiol.* **232**, H426-H433 (1977).
- Phillips, M. I., Phipps, J., Hoffman, W. E. & Leavitt, M. *Physiologist* **18**, 350 (1975).
- Sweet, C. S., Columbo, J. C. & Gaul, S. K. *Fedn Proc.* **35**, 1056 (1976).
- Schoelkens, B. A. *J.R.C.S. Med. Sci.* **4**, 320-321 (1976).
- Elghozi, J. L. *et al. Clin. Sci. molec. Med.* **51**, 385s-389s (1976).
- Fuxe, K., Ganten, D., Hökfelt, T. M. & Bolme, P. *Neurosci. Lett.* **2**, 229-234 (1976).

## Thyroid-stimulating autoantibody production *in vitro*

GRAVES' disease is an autoimmune disorder in which hyperthyroidism is associated with the presence of thyroid-stimulating autoantibodies<sup>1</sup>. Recent work suggests that these antibodies, collectively termed TSAb, are antibodies to the thyrotrophin (TSH) receptor which stimulate the thyroid by binding to the receptor and activating adenylate cyclase<sup>1-5</sup>. Synthesis of the autoantibody *in vivo* may result from defects in both the thyroid and the immune system. In the case of the thyroid, this may be an aberration in the synthesis or shedding of plasma membrane components related to the TSH receptor. In the case of the immune system, a current view is that autoantibody formation is the result of a suppressor T cell defect<sup>6</sup>. An *in vitro* model of TSAb synthesis would permit an analysis of the role of the thyroid and the immune system in the events leading to thyroid-stimulating antibody production. Attempts have been made to study TSAb production in short-term cultures of peripheral blood lymphocytes from Graves' patients.



**Fig. 1** Kinetics of IgG production by peripheral blood lymphocytes. Lymphocytes were separated on a Ficoll-Hypaque gradient<sup>19</sup> and washed three times; the yield from heparinised blood averaged 80%. The cells were incubated at  $1.0-1.5 \times 10^7$  per ml in 1 ml aliquots in Marbrook flasks with an outer reservoir of 25 ml. The culture medium was RPMI 1640 buffered with  $\text{NaHCO}_3$  and supplemented with L-glutamine (2 mM), penicillin ( $50 \text{ U ml}^{-1}$ ), streptomycin ( $50 \text{ } \mu\text{g ml}^{-1}$ ) and 10% heat inactivated foetal calf serum (Flow Laboratories, Lot 428136). Lymphocytes were incubated at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  in air for intervals of up to 28 d and collected as follows: the cell suspensions were removed from the Marbrook flasks, centrifuged and the culture supernatants separated from the cell pellets. Soluble material was extracted from the cell pellets by freezing, thawing and homogenising; the homogenates were centrifuged ( $100,000g$  for 30 min) and the supernatants (termed the soluble cell extract) collected. IgG was measured in the culture supernatants and soluble cell extracts by a solid phase radioimmunoassay<sup>20</sup>. Each kinetic curve is derived from the lymphocytes separated from 100 ml of blood from a single donor; each point is the mean of duplicate or triplicate cultures. Results are expressed as  $\mu\text{g}$  IgG produced per  $10^7$  cells initially added to each flask. ● and ○, IgG in the supernatants from cultures stimulated with PWM (Gibco,  $10 \text{ } \mu\text{l ml}^{-1}$ ); ▲ and △, IgG in cell extracts of cultures stimulated with PWM; ■ and □, IgG in the supernatants of cultures without mitogen.

These studies, however, did not use optimal conditions for immunoglobulin production and the levels of TSAb reported were close to the limits of detectability of the imprecise assay methods used<sup>7-11</sup>. In the study described here we have used the optimal conditions for culturing lymphocytes provided by Marbrook flasks<sup>12</sup> in conjunction with two new sensitive and precise methods for detecting TSAb—the radio-receptor<sup>13</sup> and cytochemical assays<sup>14,15</sup>. With this system we have readily been able to detect TSAb production in cultures of Graves' lymphocytes.

Thyroid-stimulating antibodies are always associated with the IgG fraction of serum. Therefore in preliminary experiments a study of the kinetics of IgG production *in vitro* was made using peripheral blood lymphocytes from normal donors. Lymphocytes were cultured for up to 28 d in medium alone or medium containing pokeweed mitogen (PWM) and the IgG in the culture supernatants measured by solid phase radioimmunoassay. Also, the amount of immunoglobulin remaining in the cells was determined. Some representative experiments are shown in Fig. 1. After 10 d of culture in the presence of PWM the medium contained about  $100 \text{ } \mu\text{g}$  of IgG per  $10^7$  cells and this was increased to  $200 \text{ } \mu\text{g}$  after 21 d. The cell extracts contained far less IgG. In the absence of PWM, only small

**Table 1** TSAb activity in supernatants from lymphocyte cultures measured by cytochemical assay

Culture conditions	Patient	IgG dose ( $\mu$ g)	% Stimulation	Donor	IgG dose ( $\mu$ g)	% Stimulation
Membranes + PWM	Bu	0.64	35.7	Bo	0.22	0.4
PWM	Ca	0.88	28.6	Pe	0.60	2.9
PWM	Gl	0.82	38.0	Sm	0.56	2.2

Lymphocytes were incubated with PWM, alone or after an initial 24-h period with thyroid membranes. Immunoglobulins in the culture supernatants and medium alone were precipitated using ammonium sulphate, dialysed exhaustively against isotonic saline and assayed in the section assay for thyroid stimulators<sup>21</sup>. The results are expressed as % stimulation of the absorbance (*A*) over control values (medium only) calculated from the formula:

$$[(A_{\text{test sample}} - A_{\text{control}})/A_{\text{control}}] \times 100$$

Each absorbance value is the mean of 10 measurements per slide on duplicate slides. The amount of IgG used for each assay is given. All three Graves' patients studied had high levels of serum TSAb activity.

amounts of IgG were detected in the medium and cell extracts. The ability of PWM to stimulate immunoglobulin production in human lymphocyte cultures is well known<sup>16,17</sup>. On the basis of these experiments it was decided to investigate thyroid-stimulating antibody production by culturing Graves' lymphocytes with PWM and assaying the culture supernatants for TSAb after 14 d. This time interval was chosen so as to provide near-maximal amounts of IgG while reducing the risk of infection which is a problem with long-term cultures.

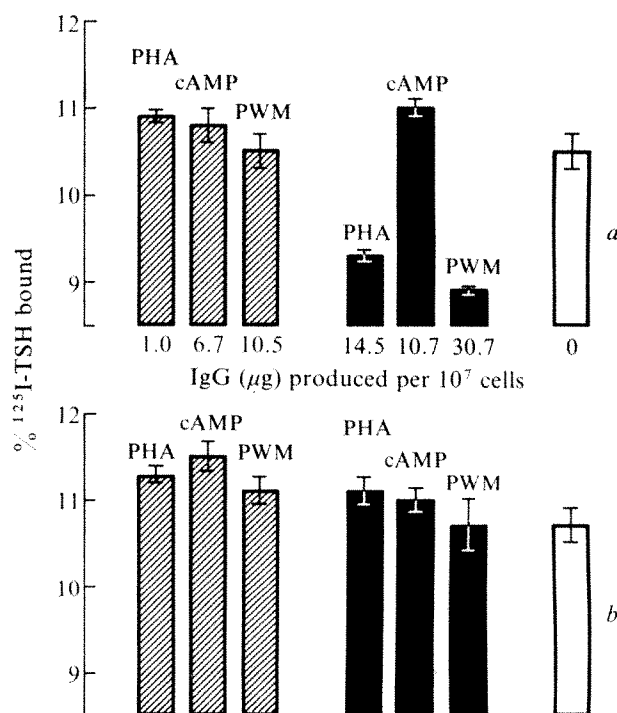
Table 1 shows the measurement of TSAb activity by cytochemical assay in the immunoglobulin fraction of the culture supernatants from Graves' and normal peripheral blood lymphocytes. The assay depends on detecting TSAb- (or TSH)-induced increases in the permeability of thyroid cell lysosomes. These increases are monitored by using a chromogenic substrate (leucyl- $\beta$ -naphthylamide) for the lysosomal enzyme naphthylamidase and the intensity of the resulting intracellular colour is quantitated by scanning and integrating microdensitometry. The supernatants from all three Graves' lymphocyte cultures produced an increase in absorbance of 30–40% over control (medium only) whereas the responses elicited by supernatants from cultures of normal lymphocytes were less than 3%. This study, in which the assay of the supernatants from cultures of normal and Graves' lymphocytes was carried out 'blind', provided good evidence that TSAb was produced by the cultured Graves' lymphocytes.

Confirmation of the presence of TSAb in Graves' lymphocyte cultures was provided by studies with the radioreceptor assay for thyroid-stimulating antibodies which depends on the ability of TSAb to inhibit the binding of labelled TSH to thyroid membranes<sup>1,3-5,13</sup>. In these experiments, lymphocytes were cultured with antigen (in the form of thyroid membranes) for 24 h. One-third of the cells were also treated with dibutyryl cyclic AMP during this period since there is evidence from experiments with mice that cyclic AMP administered with antigen *in vivo* and *in vitro* results in higher numbers of antibody-forming cells<sup>18</sup>. After 24 h, the cells were washed and re-suspended in medium alone (for cyclic AMP-treated cells) or medium plus PWM or phytohaemagglutinin (PHA). The supernatants from 14-d cultures were assayed for IgG and the immunoglobulins of the supernatants monitored for TSAb activity in the receptor assay. A significant inhibition of labelled TSH binding to thyroid membranes was observed with supernatants from Graves' lymphocytes cultured with PHA (Student's *t* = 24.5, *P* < 0.001) and PWM (*t* = 7.1, *P* < 0.01) and this indicated the presence of TSAb (Fig. 2a). The specificity of the effect of the cultures in the receptor assay was demonstrated by the experiment shown in Fig. 2b in which supernatants from the same cultures were pre-incubated with thyroid membranes before assay; the TSAb activity originally present in the PHA and PWM cultures was absorbed out.

These observations indicated that peripheral blood lymphocytes from patients with Graves' disease are capable of making thyroid-stimulating autoantibodies when cultured under the conditions provided by Marbrook flasks<sup>22</sup>. It should now be possible to use the combination of the Marbrook culture

system with the precise and sensitive cytochemical and radio-receptor assays to investigate the events leading to TSAb production. Similarly, autoantibody production in other human autoimmune diseases could be studied using this approach.

We thank Drs Chayen and Bitensky, from the Kennedy Institute of Rheumatology, for considerable help with the cytochemical bioassay. We also thank Drs J. G. Pierce and J. Fawcett and the Armour Pharmaceutical Company for gifts of TSH, Professor J. Owen, Professor A. L. Latner, Professor



**Fig. 2** TSAb activity in supernatants of lymphocyte cultures measured by radioreceptor assay<sup>13</sup>. Lymphocytes were incubated with thyroid membranes (35  $\mu$ g protein ml<sup>-1</sup>) for 24 h, washed and then cultured with PHA (Wellcome 10  $\mu$ l ml<sup>-1</sup>), PWM (10  $\mu$ l ml<sup>-1</sup>) or medium only for 14 d; cells receiving medium only had been stimulated with dibutyryl cyclic AMP (cAMP, 0.4 mM) during the 24 h incubation with membranes. IgG was measured in the culture medium and the amount produced per 10<sup>7</sup> cells is shown. Immunoglobulins were precipitated from the culture supernatants with ammonium sulphate, dialysed exhaustively against 50 mM NaCl, 10 mM Tris-HCl (pH 7.4) and assayed in the radioreceptor assay. The figure shows the effect of the immunoglobulins on <sup>125</sup>I-TSH binding to thyroid membranes. *a*, Immunoglobulins assayed directly. *b*, Immunoglobulins pre-incubated with human thyroid membranes. All values are the means of triplicate  $\pm$  s.e.m. Data are given for a Graves' patient (solid bars), a normal donor (cross-hatched bars) and culture medium alone (open bars). The same number of cells were cultured from the Graves' patient and the normal donor. The Graves' patient studied in this experiment had particularly high levels of serum TSAb; 1  $\mu$ g of purified serum IgG was readily detectable in the receptor assay.

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- Hall, R., Rees Smith, B. & Mukhtar, E. D. *Clin. Endocr.* **4**, 213-230 (1975).
- Mukhtar, E. D., Rees Smith, B., Pyle, G. A., Hall, R. & Vice, P. *Lancet* **i**, 713-720 (1975).
- Fayet, G. *et al.* *FEBS Lett.* **32**, 299-302 (1973).
- Manley, S. W., Bourke, J. R. & Hawker, R. W. *J. Endocr.* **61**, 437-445 (1974).
- Mehdi, S. Q. & Nussey, S. S. *Biochem. J.* **145**, 105-111 (1975).
- Allison, A. C. & Denman, A. M. *Br. med. Bull.* **32**, 124-129 (1976).
- McKenzie, J. M. & Gordon, J. in *Current Topics in Thyroid Research* (eds Cassano, C. & Andreoli, M.) 445 (Academic, New York, 1965).
- Miyai, K., Fukuchi, M., Kumahara, U. & Abe, H. *J. clin. Endocr. Metab.* **27**, 855-860 (1967).
- Wall, J. R., Good, B. F., Forbes, I. J. & Hetzel, B. S. *Clin. exp. Immun.* **4**, 555-561 (1973).
- Knox, A. J. S., von Westarp, C., Row, V. V. & Volpé, R. *J. clin. Endocr. Metab.* **43**, 330-337 (1976).
- Knox, A. J. S., von Westarp, C., Row, V. V. & Volpé, R. *Metabolism* **25**, 1217-1223 (1976).
- Marbrook, J. *Lancet* **ii**, 1279-1281 (1967).
- Rees Smith, B. & Hall, R. *Lancet* **ii**, 427-431 (1974).
- Bitensky, L., Alaghband-Zadeh, J. & Chayen, J. *Clin. Endocr.* **3**, 363-374 (1974).
- Petersen, V., Smith, B. R. & Hall, R. *J. clin. Endocr. Metab.* **41**, 199-202 (1975).
- Wu, L. Y. F., Lawton, A. R. & Cooper, M. D. *J. clin. Invest.* **52**, 3180-3189 (1973).
- Janossy, G., Gomez de la Concha, E., Waxdal, M. J. & Platts-Mills, T. *Clin. exp. Immun.* **26**, 108-117 (1976).
- Weinstein, Y. & Melmon, K. L. *Immun. Comm.* **5**, 401-416 (1976).
- Boyum, A. *Scand. J. Immun.* **5** suppl. 5, 9-15 (1976).
- Wide, L., Nillius, S. J., Gemzell, C. & Roos, P. *Acta Endocr. suppl.* **174**, 1-58 (1974).
- Gilbert, D. M., Besser, G. M., Bitensky, L. & Chayen, J. *J. Endocr.* (in the press).
- North, J. R. & Maizeis, R. M. *Immunology* **32**, 771-776 (1977).

## Relaxin has conformational homology with insulin

RELAXIN, a polypeptide hormone synthesised and stored in the corpus luteum, is responsible for the dilation of the symphysis pubis in most mammals before parturition. Porcine relaxin (molecular weight ~5,600) consists of one A chain and one B chain linked by disulphide bonds. The amino acid sequence of the two chains is consistent with interchain and intrachain disulphide crosslinks of the same disposition as those of insulin<sup>1,2</sup>. Although only five further residues are identical to those in equivalent positions of porcine insulin, the model presented here shows that relaxin may have a tertiary structure closely resembling that of

insulin. The residues of the hydrophobic core of relaxin differ from those of insulin but are close packed and occupy the same volume.

Figure 1 shows the sequence of porcine relaxin as determined by Schwabe *et al.*<sup>1,2</sup> aligned with the sequence of porcine insulin according to the positions of the cystines (for reviews see refs 3, 4). In this paper the insulin numbering scheme has been applied to the relaxin sequence to facilitate comparison of the equivalent residues in the two molecules. Some relaxin molecules apparently have additional residues, possibly serine and arginine at the C-terminus of the B chain<sup>2</sup>. This may, by analogy with insulin, be a consequence of there being a precursor, prorelaxin; these derivatives may be intermediates in the conversion process. A further sequence analysis of a porcine relaxin by Niall and co-workers<sup>5</sup> shows that there may be some heterogeneity in relaxin. Since these differences seem to be limited to residues at the B-chain termini located on the outside of the model presented here, they could be accommodated without affecting the relaxin tertiary structure that we describe.

We have explored a possible three-dimensional structural homology of insulin and relaxin in the following way. A model was built first of the main chain A1-A20 and B6-B21, in which the interchain and intrachain disulphide bridges occupied equivalent three-dimensional positions to those reported for insulin, and other amino acid residues shown to be equivalent in the sequence alignment of Fig. 1 were given the same main chain conformation. This was made possible by the presence of glycines at B8 and B20 in relaxin as in insulin, in which positions an amino acid with a side chain would not be allowed as a consequence of the positive  $\psi$  angles. The side chains of B6, B11, B12, B14, B15, B18, A2 and A16 which contribute to the hydrophobic core of insulin were then built into the framework provided by the main chain and cystine disulphides. These are all hydrophobic in relaxin and are easily accommodated to give a close packed core. B11 and B12 are conserved as in all insulins. Of particular interest is the complementary nature of many of the sequence differences. For example, in insulin B6 Leu and B14 Ala are in close juxtaposition; in relaxin their respective positions are reversed: B6 Ala and B14 Leu. In a similar way A2 Ile and A16 Leu of insulin are A2 Leu and A16 Ile in relaxin. These pairs of side chains point towards the centre of the core from opposite sides. The close-packed occupancy of the core is thus maintained. The B15 Trp of relaxin is beautifully accommodated almost completely buried in the core with no relative changes of the positions of the A and B chains. The proposed main chain conformation of relaxin is shown in Fig. 2 and a stereoview of the three-dimensional structure is shown in Fig. 3.

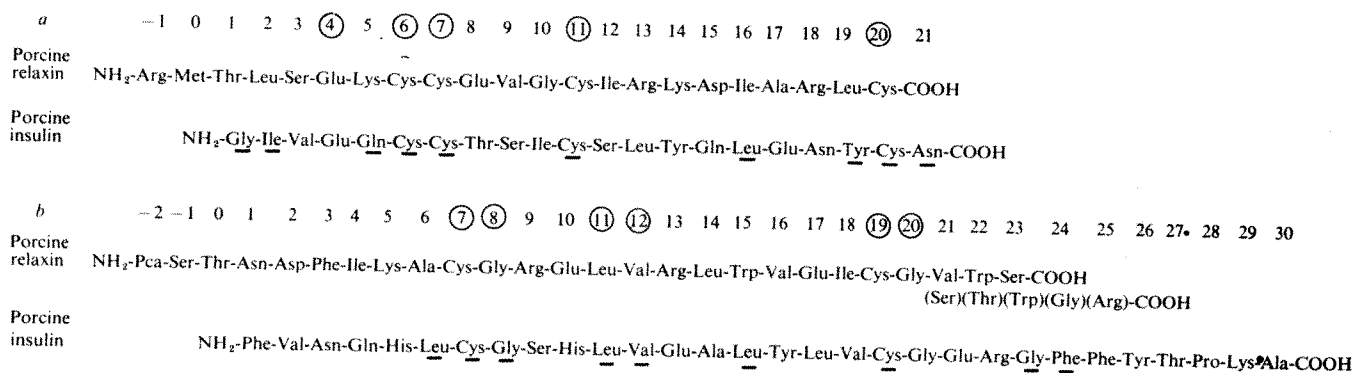
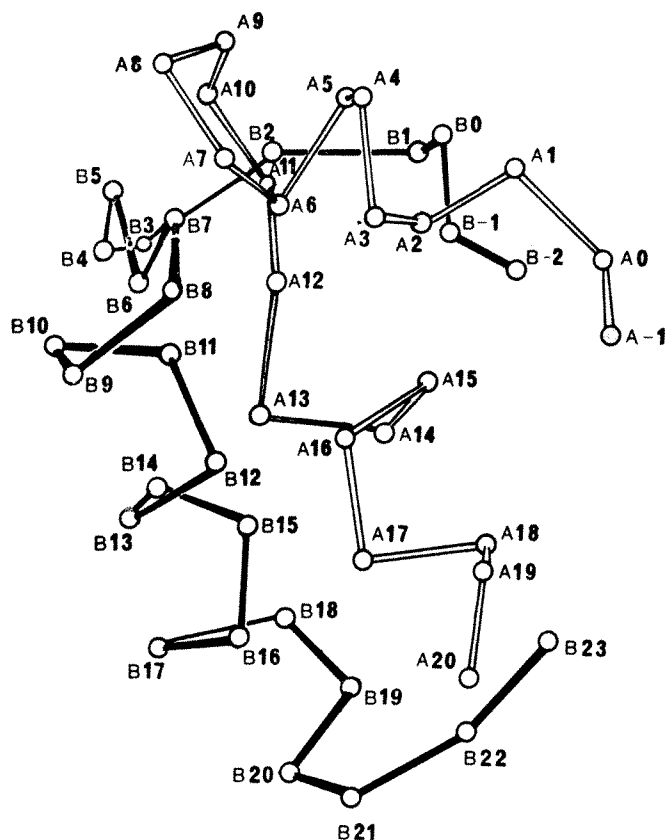


Fig. 1 Sequences of porcine relaxin<sup>1,2</sup> and insulin arranged to give alignment of the cystines. There may be further Ser and Arg residues at B24 and B25 in some relaxin molecules<sup>2</sup> and an independent sequence determination<sup>5</sup> indicates possible heterogeneity at B-2, B21, B22 and B23 (the sequence differences are given in parentheses). The numbers of residues identical in porcine insulin and relaxin are circled and residues invariant in all known insulins are underlined.





**Fig. 2** Proposed conformation of the relaxin main chain indicated by  $\alpha$  carbon positions ( $\circ$ ) and joined by virtual bonds. The residue numbers are those shown in Table 1 to facilitate comparison with insulin. The molecule is viewed from a position equivalent to right angles to the three-fold axis and into the face involved in dimerisation of the insulin hexamer (see Fig. 10 of ref. 3). In insulin the B chain continues from B23 to a position close to A2 partly covering the A chain residues as viewed here.

Having established the feasibility of an insulin-like fold for relaxin we then proceeded to add surface residues including the chains B6–B22 and A1–A20. This naturally led to the suggestion of many ion pairs in particular A5 Lys and A15 Asp of relaxin, which are A5 Gln and B15 Gln in insulin. Further possible relaxin ion pairs are A8 Glu and B5 Lys, B13 Arg and B17 Glu.

Residue A19 is Tyr in insulin and Leu in relaxin. This change is easily accommodated in the model and may be related to the extra residues at the A-chain N terminus of

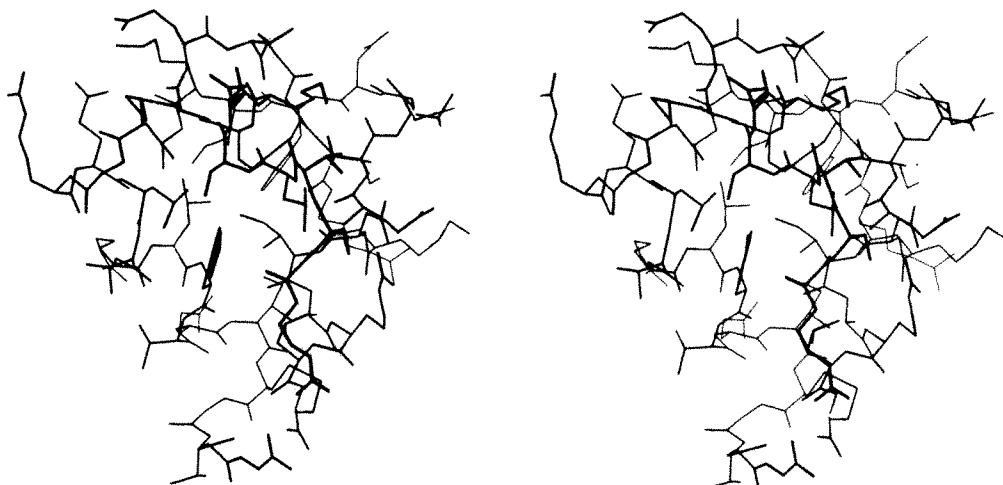
relaxin which are most probably covering A19 so that A0 Met is in contact with Leu, leading to a further ion pair A–1 Arg to the B-chain C terminus. In a similar way the extra residues at the B-chain N terminus are easily accommodated. When residue B3 Phe was allowed to lie against the residues of the hydrophobic core, the chain could be further extended to fold over A12, a position which is Ile in relaxin although always hydrophilic in insulins. But, the conformations of the B-chain N terminal residues may be flexible in relaxin as they are in insulin, and it is possible that they contribute little to the stability of the insulin-like core. The absence of a residue at A21 might be expected to destabilise the insulin-like tertiary structure, but would not prevent its existence.

Although of less consequence for the tertiary structure of relaxin, the positioning of the residues at the B-chain C terminus must await clarification of this part of the primary structure. If the polypeptide chain has either of the reported sequences<sup>2,3</sup>, it can be folded back on to the hydrophobic core. The tryptophan probably contributes towards stabilising the tertiary structure. This residue may be placed so that it partially compensates for the lack of residues in relaxin equivalent to B24 Phe and B26 Tyr of insulin. Thus the tertiary structure would be expected to be more stable than that of desoctapeptide insulin (B23–B30 deleted) which has a tertiary structure rather different from that of native insulin. Residue A3 in our model of relaxin—a serine residue—occurs on the surface of the molecule whereas in insulin this residue is usually a valine and is close to the B-chain C terminus.

The uncertainty in the C-terminal B-chain sequence may be due to the existence of two variants of relaxin suggesting that this portion of the molecule makes only a limited, contribution, if any, to the biological activity. Thus, the tryptophan residue known to be inessential<sup>6</sup> is most likely the one located in the C-terminal tail of the relaxin B chain. Our model predicts that the remaining tryptophan B15 which is buried cannot be oxidised in native relaxin without loss of tertiary structure and probably therefore biological activity. The relative mobility of the C-terminal peptide of the B-chain may be deduced from the fact that carboxypeptidase A digestion of native relaxin readily releases Ser, Trp, Val, and somewhat more slowly, Gly.

The absence of histidine at B10 implies that relaxin will not bind zinc ions to give a hexamer in the same way as insulin, although the B10 Glu of relaxin may bind other metal ions such as calcium. But, the association of the protomers would also be hindered by the change of the C-terminus of the relaxin B-chain and the presence of charged groups at B17 and A13 in relaxin which are usually hydrophobic in insulin. The equivalent groups in

**Fig. 3** A stereo view of the relaxin model from approximately the same direction as Fig. 2. All side-chain residues are included with the exception of those of B21, B22 and B23. The tryptophan (B15) is seen centrally end on and partially buried in the hydrophobic core. The drawing is taken from coordinates measured from the model and constrained towards the known geometry of amino acids using the method described in ref. 9.



insulin are involved in protomer-protomer interactions in the zinc insulin hexamer.

The differences between residues at A1 and A19 and the deletions at the two chain termini make it unlikely that relaxin will bind insulin receptors, since modification or deletion of these residues in insulin leads to considerable loss of affinity for the receptor and a decrease of biological activity<sup>3,7</sup>.

Relaxin in its highly purified form requires an adjuvant such as beeswax or benzopurpurin 4- $\beta$  for enhancement of biological activity. This may well be due to interaction of the diazo dye, for example, with the hydrophobic residues, particularly tryptophan B22 and subsequent stabilisation of the structure and protection from proteolytic attack. The induction of a strong Cotton effect at 520 nm indicates that the dye molecules bind relaxin strongly and specifically (data not shown).

The existence of a molecule with the sequence of relaxin and an insulin-like tertiary structure may be explained by gene duplication of primitive insulin-like molecules followed by accepted mutations leading to radical changes in sequence<sup>8</sup>. If this is so, the nature of the complementary changes in the hydrophobic core implies evolution through a less easily folded state possibly implying a period without selective pressure on the duplicated gene.

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*Note added in proof:* An independent model building study using computer graphics has also concluded that relaxin has an insulin-like conformation (N. Isaacs *et al.*, manuscript in preparation).

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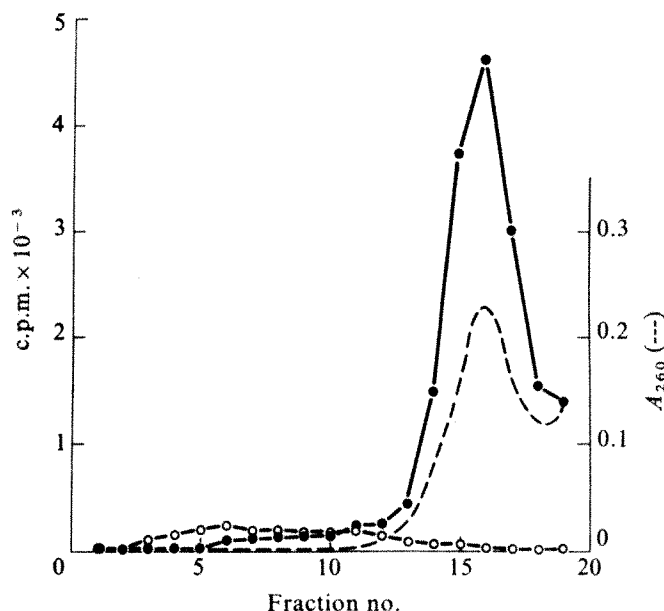
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- Schwabe, C., McDonald, J. K. & Steinetz, B. G. *Biochem. biophys. Res. Commun.* **70**, 397-405 (1976).
- Schwabe, C., McDonald, J. K. & Steinetz, B. G. *Biochem. biophys. Res. Commun.* **75**, 503-510 (1977).
- Blundell, T. L. *et al. Adv. Protein Chem.* **26**, 280-394 (1972).
- Blundell, T. L. & Wood, S. P. *Nature* **257**, 197-203 (1975).
- Kwok, S., Bryant-Greenwood, G., James, R. & Niall, H. *Nature* **267**, 544-546 (1977).
- Schwabe, C. & Braddon, S. A. *Biochem. biophys. Res. Commun.* **68**, 1126-1132 (1976).
- Pullen, R. A. *et al. Nature* **259**, 369-373 (1976).
- Schwabe, C. & McDonald, J. K. *Science* (in the press).
- Dodson, E. J., Isaacs, N. W. & Rollett, J. S. *Acta crystallogr.* **A32**, 311-315 (1975).

## Nucleosome structure controls rates of excision repair in DNA of human cells

EUKARYOTIC chromatin is now considered to be composed of structural units, nucleosomes (nu bodies), which consist of four pairs of histones around which DNA is coiled<sup>1-3</sup>. Approximately 200 base pairs of DNA are associated with each nucleosome, 140 base pairs are wrapped around each and a variable length of approximately 40 base pairs lies between<sup>1-3</sup>. The DNA wrapped around each nucleosome is less easily digested by micrococcal nuclease than the DNA between them, and brief periods of incubation with the enzyme allow isolation of nucleosomes



**Fig. 1** Repair replication in confluent human fibroblasts. Cultures were incubated in the presence of BUdr  $10^{-5}$  M and FUdr  $2 \times 10^{-6}$  M for 1 h, irradiated with  $13 \text{ J m}^{-2}$  ultraviolet light and labelled for 2 h in BUdr  $10^{-5}$  M,  $^3\text{H}$ -Tdr  $10 \mu\text{Ci ml}^{-1}$  (64 Ci  $\text{mmol}^{-1}$ ), FUdr  $2 \times 10^{-6}$  M, hydroxyurea  $2 \times 10^{-3}$  M; the DNA was isolated and sedimented in alkaline caesium chloride gradients<sup>10,11</sup>. The exclusive location of  $^3\text{H}$  label at normal density (coincident with the absorbance peak) indicates that the  $^3\text{H}$  has been incorporated by repair replication. Broken line,  $A_{260}$ ;  $\circ$ , controls;  $\bullet$ , irradiation with  $13 \text{ J m}^{-2}$  ultraviolet. Radioactivity profiles were normalised to the same amount of DNA per gradient.

as discrete particles<sup>2</sup>. The structural organisation of mammalian DNA into nucleosomes, and higher degrees of order in the packing of strings of nucleosomes into chromosomal fibres has been shown to play a major part in the control of DNA replication<sup>4,5</sup>, and transcription<sup>6</sup>. Previous studies have indicated that the accessibility of damage in DNA to repair enzymes is restricted by chromatin proteins<sup>7,8</sup> and it is therefore likely that the repair of damaged sites in DNA is also controlled by some features of nucleosome structure and packing<sup>9</sup>. In the study described here I have measured the rates of degradation of repaired regions in mammalian DNA by micrococcal nuclease; the results suggest that the first sites of ultraviolet light-induced damage to be repaired are those in the DNA between nucleosomes and that there is little rearrangement of nucleosomes along the DNA during repair.

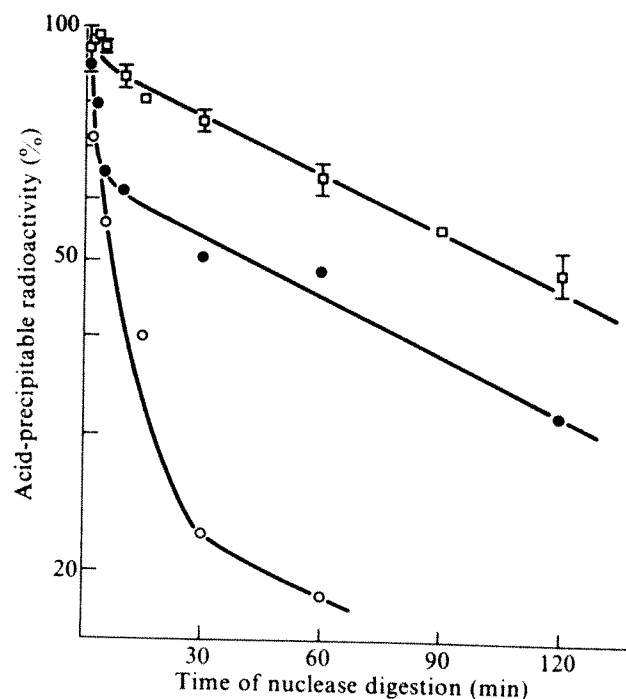
Human fibroblast cultures (strain E11) were established from foreskin and grown in Eagle's minimal essential medium with 10% foetal calf serum. Cultures for experiments were grown in  $0.01 \mu\text{Ci ml}^{-1}$ ,  $56.4 \text{ mCi mmol}^{-1}$   $^{14}\text{C}$ -thymidine ( $^{14}\text{C}$ -Tdr) in 90-mm Petri dishes until confluent and mitotic figures no longer detectable. Cultures were then rinsed and grown for 24 h in unlabelled medium before being once irradiated with  $13 \text{ J m}^{-2}$  ultraviolet light (254 nm, at a dose rate of  $1.3 \text{ J m}^{-2} \text{ s}^{-1}$ ). They were then labelled with  $^3\text{H}$ -Tdr ( $10 \mu\text{Ci ml}^{-1}$ ,  $64 \text{ Ci mmol}^{-1}$ ), hydroxyurea ( $5 \times 10^{-3}$  M), and fluorodeoxyuridine (FUdr,  $2 \times 10^{-6}$  M) for 10 min to 4.5 h. For most experiments cells were collected immediately but some were allowed to grow in  $5 \times 10^{-4}$  M Tdr and  $5 \times 10^{-5}$  M deoxycytidine for several hours before collection.

Confluent cultures of primary fibroblasts perform negligible amounts of semiconservative DNA replication, and all of the radioactivity incorporated into DNA in the presence of hydroxyurea after ultraviolet irradiation consists of repair replication (Fig. 1). Therefore, in cultures allowed to reach confluency in  $^{14}\text{C}$ -Tdr, irradiated with ultraviolet light and labelled with  $^3\text{H}$ -Tdr, the  $^{14}\text{C}$  activity is uniformly distributed throughout the DNA but the  $^3\text{H}$  activity is confined to the short patches which are involved in repair of ultraviolet damage in DNA<sup>10,11</sup>.

Repaired regions in cells that had incorporated  $^3\text{H}$ -Tdr immediately after ultraviolet irradiation were more rapidly digested by nuclease action than was  $^{14}\text{C}$ -Tdr-labelled DNA (Fig. 2, Table 1). When cells that had incorporated  $^3\text{H}$ -Tdr into repaired DNA immediately after irradiation had grown for 5 h, the  $^3\text{H}$  label was digested to a similar extent to that in cells collected earlier (Fig. 2, Table 1). When cells were labelled for longer periods the proportion of  $^3\text{H}$  activity that was rapidly digested decreased (Fig. 2, Table 1).

These results show that the rate at which nuclease degrades  $^3\text{H}$ -labelled DNA in repaired regions is different from that at which  $^{14}\text{C}$  uniformly labelled DNA is degraded. To a first approximation, the degradation kinetics can be analysed in terms of two exponential components (Fig. 2): an initial rapid component which is present in decreasing proportion as the labelling time increases, and a slow component which has approximately the same slope at all labelling times (Fig. 2, Table 1). The simplest interpretation of these results is that the rapid component represents degradation of the DNA between nucleosomes, and the slow component represents degradation of DNA closely associated with nucleosomes. This interpretation is strengthened by observation that the DNA after 5 min digestion with nuclease in essentially the same conditions in this laboratory is in one or more multiples of approximately 200 nucleotide pair lengths (B. Young, unpublished). The results indicate therefore, that at short times after irradiation the repaired regions are predominantly between nucleosomes, but after longer times all regions of DNA contain repaired regions.

There are two possible reasons for the change in the distribution of  $^3\text{H}$  label in repaired regions with increasing labelling times. One is that only internucleosome DNA is repaired and that there is a redistribution of nucleosomes along the DNA which tends to randomise the label. The other is that internucleosome DNA is more accessible to repair enzymes and is repaired more rapidly than DNA on the nucleosomes. Because the inter-nucleosome DNA and hence the ultraviolet-induced photoproducts in this DNA are a minor fraction of the whole DNA and a minor fraction of the total repair replication is completed in the first hour after irradiation (Table 1), faster repair in these regions will result in earlier completion of repair; at longer times repair on nucleosomes would predominate. The first possibility is eliminated, however, by the pulse-chase experiments. Growing cells for 5 h after a 30-min pulse of  $^3\text{H}$ -Tdr immediately following irradiation produced a  $^3\text{H}$  distribution that was similar to that obtained from similarly labelled cells collected earlier (Table 1). Cells that had been labelled for only 10 min, however, had a  $^3\text{H}$  distribution in which the rapidly digested component decreased from 58% to 39%. These observations at 10 and 30 min of labelling indicate that the distribution of DNA between and on nucleosomes changes little during 5 h of growth. The slight change observed in the 10-min labelled sample could actually be due to variability



**Fig. 2** Percentage of  $^{14}\text{C}$ - or  $^3\text{H}$ -labelled DNA in nuclei remaining undigested by micrococcal nuclease after various times of incubation at  $37^\circ\text{C}$ . After cultures were labelled and irradiated as described in the text, cells were collected by rinsing once in physiological buffer, trypsin treatment for 2 min at  $37^\circ\text{C}$  and the suspension was centrifuged at  $1,800g$  for 1 min. Cells were resuspended in ice-cold hypotonic buffer (RSB,  $0.0015\text{ M MgCl}_2$ ,  $0.015\text{ M NaCl}$ ,  $0.01\text{ M Tris-HCl}$ ,  $\text{pH } 7.4$ ) and centrifuged again for three successive washes. They were resuspended in RSB containing  $1\%$  Triton X-100 and gently homogenised by 10 strokes in a tight fitting Dounce homogeniser to prepare nuclei<sup>9</sup>. Nuclei were centrifuged for 1 min at  $1,800g$  and then resuspended in RSB and centrifuged three more times. The nuclear pellet was resuspended in RSB,  $\text{CaCl}_2$  added to a final concentration of  $10^{-3}\text{ M}$  and the suspension dispensed in  $0.4\text{ ml}$  quantities into tubes containing  $2\text{ }\mu\text{l}$  of micrococcal nuclease (Worthington, 40 units of enzyme to approximately  $10^4$  nuclei), and incubated at  $37^\circ\text{C}$ . Individual tubes were removed at various times, chilled on ice,  $100\text{ }\mu\text{g}$  carrier DNA added and the DNA precipitated with perchloric acid (PCA) at a final concentration of  $5\%$ . The supernatant was counted in  $7\text{ ml}$  water miscible liquid scintillation fluid (Aquasol). The precipitate was dissolved in  $5\%$  PCA at  $90^\circ\text{C}$  and counted in Aquasol. The fractions of  $^{14}\text{C}$  and  $^3\text{H}$  radioactivity rendered acid soluble by micrococcal nuclease was calculated from the radioactivity in supernatant and precipitate fractions from each tube.  $\square$ , Mean and standard error of  $^{14}\text{C}$ -Tdr uniformly-labelled cells.  $\circ$ , Labelled for 10 min with  $^3\text{H}$ -Tdr immediately after  $13\text{ J m}^{-2}$  ultraviolet light.  $\bullet$ , Labelled for 30 min immediately after  $13\text{ J m}^{-2}$  ultraviolet light.

**Table 1** Percentage of total radioactivity in the rapidly digested components of DNA in nuclei digested with micrococcal nuclease

Labelling and irradiation conditions	% Rapidly digested*		Repair replication†
	10	(10)	
Uniformly labelled $^{14}\text{C}$ -Tdr			—
Repaired, 10 min, immediately post UV	58	(2)	4
Repaired, 30 min, immediately post UV	33	(2)	14
Repaired, 30 min, immediately post UV‡	29	(1)	14
Repaired, 90 min, immediately post UV	19	(2)	53
Repaired, 4.5 h, immediately post UV	21	(2)	120
Repaired, 30 min, immediately post UV and grown for 5 h	35	(1)	—
Repaired, 10 min, immediately post UV and grown for 5 h	39	(1)	—

\*Values were calculated by extrapolation of the slow component of parallel curves similar to those shown in Fig. 2. Each value was obtained from the average of at least two separate determinations and is therefore not necessarily the same as the values that can be read off Fig. 2 since the latter illustrates single experimental determinations at each labelling time (the number in parentheses indicates number of separate degradation curves used to estimate this percentage).

†Amounts of repair replication expressed in arbitrary units calculated from experiments similar to those of Fig. 1, done for various labelling times.

‡Labelling done with  $^3\text{H}$ -Tdr but without hydroxyurea and FUdr.  
UV, ultraviolet irradiation.

in the precise labelling duration, because the nuclease digestion kinetics change rapidly during the shorter labelling periods. The distribution of repaired regions is therefore probably due to a faster rate and earlier completion of repair in inter-nucleosome DNA than in nucleosomal DNA.

In a previous study of the repair of alkylation damage in mouse cells Bodell<sup>12</sup> also showed that repair was concentrated on inter-nucleosomal DNA. In that study, however, it could be argued that there was either a non-uniform distribution of damage or a non-uniform distribution of repair, or both, because alkylating agents do not react with all regions of DNA to equal extents<sup>13</sup>. In the present study on the repair of ultra-violet damage it is most likely that the non-uniformity I have observed is due to the mechanism of repair, because pyrimidine dimers are caused by direct absorption of quanta of ultra-violet light in pyrimidine rings and the yield of photoproducts is insensitive to DNA conformation<sup>14</sup>. Some small effect of DNA structure on dimer formation which would result in a non-random distribution of dimers within or between nucleosomes cannot be completely excluded, however, but is unlikely.

These results (Fig. 2) can be interpreted to indicate that damaged DNA between nucleosomes is more rapidly repaired than DNA on the nucleosomes, this would explain many features of DNA repair previously observed. The size of the patch that replaces excised pyrimidine dimers, for example, has been usually estimated after long labelling times<sup>15, 16</sup>, after which most of the repair would be in regions of DNA on nucleosomes. Patch sizes estimated by a variety of methods seem to be about half the single-strand length of DNA associated with one nucleosome<sup>9, 10</sup>. This is what would be predicted if a randomly located dimer in a nucleosome is the starting point for an excision repair patch and the next nucleosome is the termination point. This model also predicts that the first excision repair patches made between nucleosomes after ultra-violet irradiation should have a much smaller patch size than those made later.

Several previous observations are consistent with a model in which excision repair is controlled by the structure of chromatin. Paterson *et al.*<sup>17</sup> found that excision of dimers occurred at two rates, an initial fast rate and a later slow one, and many studies on repair replication showed similar rate changes<sup>18-20</sup>. The possibility that dimers are not all equally accessible to repair enzymes was suggested by Wilkins and Hart<sup>7</sup> who showed that those dimers that were more rapidly excised from DNA were also more accessible to an exogenously supplied *Micrococcus luteus* 'ultraviolet specific' endonuclease. Mortelmans *et al.*<sup>8</sup> also showed that several complementation groups of the human DNA repair deficient disease xeroderma pigmentosum lack cofactors that control the repair of DNA in chromatin, rather than lack repair enzymes themselves as originally thought<sup>15, 21</sup>.

I conclude from these results (Fig. 2, Table 1), that the structure of mammalian chromatin exerts restraints on the rate of excision repair of ultraviolet-induced damage. In constructing models for excision repair it should, therefore, be considered that perhaps the substrate itself is the more complex entity rather than the association of repair enzymes, which has been frequently suggested<sup>22-24</sup>. The need for controlled access to damage in DNA may be one reason for the greater complexity of repair mechanisms in eukaryotes than in prokaryotes, which is indicated by the multiple complementation groups which affect excision-repair in xeroderma pigmentosum<sup>25, 26</sup>.

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1. Noll, M. *Nature* **251**, 249-251 (1974).
2. Olins, A. L., Carlson, R. D., Wright, E. B. & Olins, D. E. *Nucleic Acids Res.* **3**, 3271-3291 (1976).
3. Oudet, P., Gross-Bellard, M. & Chambon, P. *Cell* **4**, 281-300 (1975).
4. Weintraub, H. *Cold Spring Harbor Symp. quant. Biol.* **38**, 247-256 (1973).
5. Searle, R. L. *Cell* **9**, 423-429 (1976).
6. Piper, P. W. *et al.* *Nucleic Acids Res.* **3**, 493-505 (1976).
7. Wilkins, R. J. & Hart, R. W. *Nature* **247**, 35-36 (1974).
8. Mortelmans, K., Friedberg, E. C., Slor, H., Thomas, G. H. & Cleaver, J. E. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2757-2761 (1976).
9. Cleaver, J. E. in *Molecular Human Cytogenetics ICN/UCLA Symposium* (in the press).
10. Cleaver, J. E. *Adv. Radiat. Biol.* **4**, 1-75 (1974).
11. Cleaver, J. E. *Meth. Cancer Res.* **9**, 123-165 (1976).
12. Bodell, W. J. *Nucleic Acids Res.* **4**, 2619-2628 (1977).
13. Sciudero, D. & Strauss, B. *Mutat. Res.* **35**, 311-324 (1976).
14. Patrick, M. H. & Gray, D. M. *Photochem. Photobiol.* **24**, 507-513 (1976).
15. Cleaver, J. E. *Nature* **218**, 652-656 (1968).
16. Regan, J. D., Setlow, R. B. & Ley, R. *Proc. natn. Acad. Sci. U.S.A.* **68**, 708-712 (1971).
17. Paterson, M. C., Lohman, P. H. M. & Sluyter, M. L. *Mutat. Res.* **19**, 245-256 (1973).
18. Cleaver, J. E. *Photochem. Photobiol.* **12**, 17-28 (1970).
19. Cleaver, J. E., Thomas, G. H., Trosko, J. E. & Lett, J. T. *Expl Cell Res.* **74**, 67-80 (1972).
20. Smith, C. A. & Hanawalt, P. C. *Biochim. biophys. Acta* **447**, 121-132 (1975).
21. Cleaver, J. E. *Proc. natn. Acad. Sci. U.S.A.* **69**, 428-435 (1969).
22. Haynes, R. H. *Radiat. Res. Suppl.* **6**, 232 (1966).
23. Gianelli, F. & Pawsey, S. A. J. *Cell Sci.* **15**, 163-176 (1974).
24. Ahmed, F. E. & Setlow, R. B. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1548-1552 (1977).
25. Cleaver, J. E. & Bootsma, D. A. *Rev. Gen.* **9**, 19-38 (1975).
26. Kraemer, K. H., Andrews, A. D., Barrett, S. F. & Robbins, J. H. *Biochim. biophys. Acta* **442**, 147-153 (1976).

## Anisotropy of the Young's modulus of bone

THE anisotropy in elastic deformation exhibited by compact bone sections is well known<sup>1</sup>, but has been demonstrated mainly for orientations parallel to, (designated as longitudinal) or, normal to, (designated as transverse or radial), the long axis of the bone. Various models based on the concept that bone is a hydroxyapatite-reinforced collagen composite have been proposed (for example ref. 2) to account for the elastic behaviour of compact bone, as defined by the Young's modulus ( $E$ ), but lack of data for a range of orientations has precluded one critical test of their validity. We report here preliminary measurements on the Young's modulus of mature bovine femur cortical bone for specimens orientated at various angles from 0° to 90° to the bone long axis. These results were obtained on a series of rectangular-shaped, machined, specimens (4 mm × 3 mm) with two different lengths (15 mm and 20 mm), by a novel differential ultrasonic technique, which is illustrated in Figs 1 and 2.

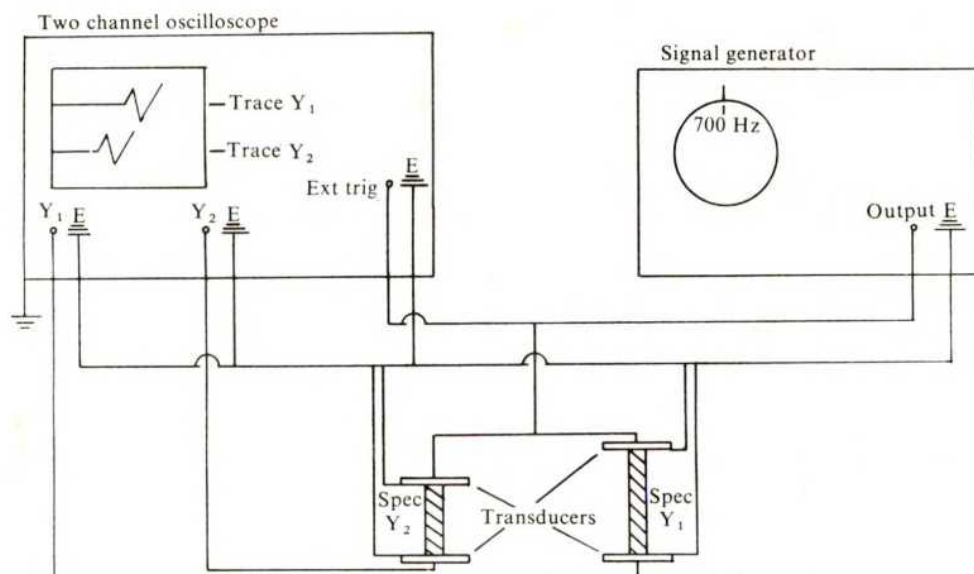
The time between the generation and receipt of a signal was measured (in  $\mu$ s) for specimens of 15 mm and 20 mm length, and as all other features of the circuit remained constant, the difference in time ( $dt$ ) for the two conditions was related to the difference in path length ( $dl$ ) provided by the bone specimens, and the velocity ( $v$ ) through the bone calculated. Assuming the path length in bone is isotropic, the Young's modulus ( $E$ ) is then derived from:—

$$\frac{dl}{dt} = v = \left( \frac{E}{\rho} \right)^{\frac{1}{2}} \quad (1)$$

where  $\rho$  is the specific gravity of bone<sup>1</sup>. This procedure was repeated for pairs of specimens orientated at various angles to the longitudinal axis (in a tangential rotation).

The absolute values of  $E$  calculated for the longitudinal (0°) orientation (17.3–19.7 GNm<sup>-2</sup>) are within the range of values (7–27 GNm<sup>-2</sup>) obtained by various workers<sup>1</sup> from the measurement of stress-strain curves, although smaller than a single value obtained on 'dry' bovine femur sections by an ultrasonic technique (26 GNm<sup>-2</sup>) (ref. 3). For the transverse orientation (90°), the calculated values of  $E$  (8.7–10.3 GNm<sup>-2</sup>) compare with values of 7.2 GNm<sup>-2</sup> (ref. 1) (stress-strain curve) and 13.2 GNm<sup>-2</sup> (ref. 4) (shock tube loading). The present results indicate that  $E_0/E_{90} = 2.3$  (dry) or 1.7 (wet), which agrees reasonably with previous findings<sup>1</sup> of a ratio of 1.9 (wet). These points of agreement suggest that the results obtained by the present technique are valid.



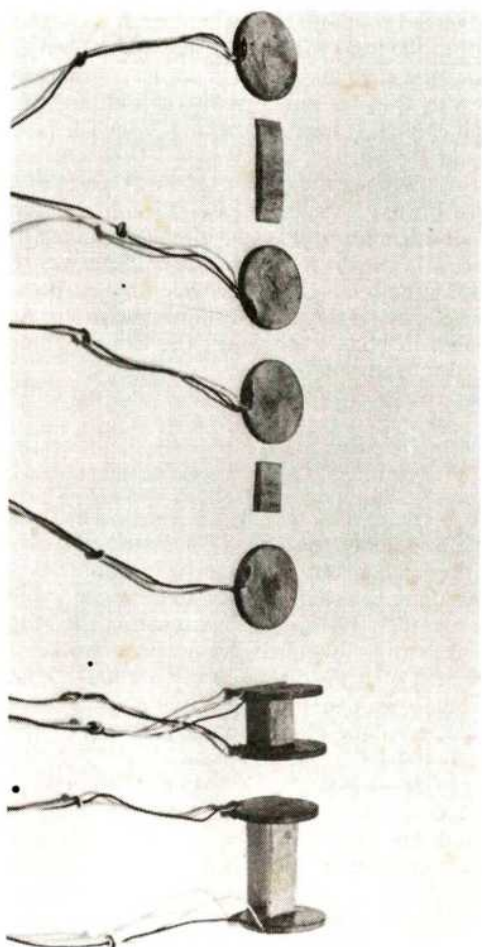


**Fig. 1** Circuit arrangement, with a signal through two specimens  $\gamma_1$  and  $\gamma_2$  producing traces  $\gamma_1$  and  $\gamma_2$ , respectively, on a two-channel oscilloscope.

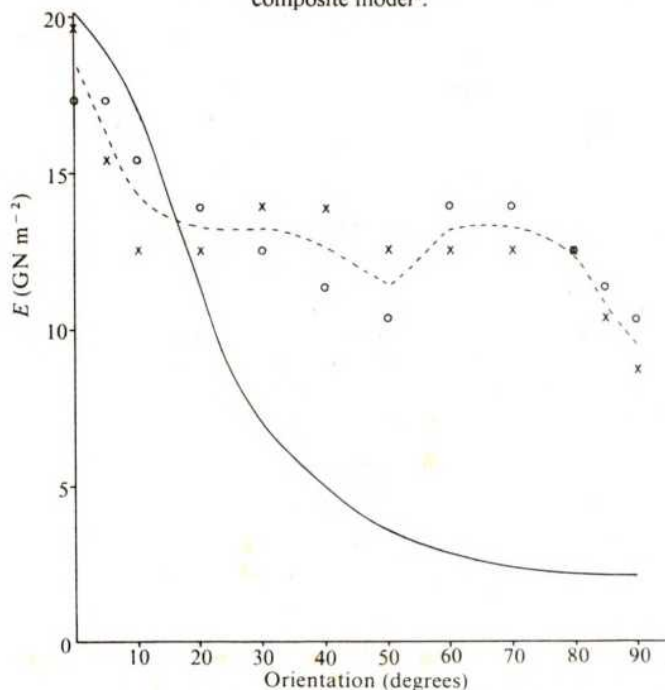
No other results have been reported for the Young's modulus of bovine femur specimens orientated at angles of 5, 10, 20, 40, 50, 70, 80 and 85° to the long axis, which are shown in Fig. 3. The Young's modulus values at 0, 30, 60 and 90° compare with values of 23.1, 16.7, 12.8 and 10.4 GNm<sup>-2</sup>, respectively, measured by Reilly and Burstein<sup>5</sup>.

The significant finding of the present study is the experimentally-determined variation in Young's modulus with orientation, which exhibits a decrease in  $E$  from 0–20°, an approximate plateau from 20–70° (with a 'low' point at 50°) and another decrease from 70–90°. This result is in conflict with the predictions of a fibre-reinforced composite model, such as demon-

**Fig. 2** Details of the experimental arrangement of transducers and bone specimens, before and after mounting. (Specimens shown are not of the experimental dimensions.)



**Fig. 3** Variation in Young's modulus of bovine femur specimens ( $E$ ) with the orientation of specimen axis to the long axis of the bone, for wet (○) and dry (×) conditions compared with the theoretical curve (—) predicted from a fibre-reinforced composite model<sup>2</sup>.



strated by the theoretical curve<sup>2</sup> (derived for  $E_{0^\circ}/E_{90^\circ} = 10$ ) plotted for comparison in Fig. 3, which reveals a progressive reduction in the dependence of Young's modulus on orientation for values of  $E$  from  $\approx 10^\circ$  to  $90^\circ$ , with an almost constant value of  $E$  in the  $70^\circ$ – $90^\circ$  range.

We conclude, therefore that an alternative model is required to account for the dependence of Young's modulus on orientation. Further work is in progress to extend these results to a series of bones with different ultrastructures.

This research forms part of a project on the deformation and fracture of bone supported by the SRC.

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- 1 Evans, F. G. *Mechanical Properties of Bone* (Charles C. Thomas, Springfield, Illinois, 1972).
- 2 Currey, J. D. *J. Biomech.* **2**, 477–480 (1969).
- 3 Lang, S. B. *Science* **165**, 287–288 (1969).
- 4 Bonfield, W. & Datta, P. K. *J. Mat. Sci.* **9**, 1609–1614 (1974).
- 5 Reilly, D. & Burstein, A. H. *J. Biomech.* **8**, 393–405 (1975).

# reviews

## Nuclear phenomena and development

A. T. Sumner

*Nuclear Cytology in Relation to Development.* By F. d'Amato. Pp. viii+283. (Cambridge University: New York, London and Cambridge, 1977.) £15.

THE illustration on the jacket of this book of Balbiani rings on a polytene chromosome seems particularly appropriate for the subject of *Nuclear Cytology in Relation to Development*. In fact, Professor d'Amato takes his subject matter from a much wider field than this illustration might suggest. "Nuclear cytology" is regarded as ranging from molecular biology to cytogenetics; what one might regard as nuclear cytology in the strict sense—the detailed study of nuclear structure—does not occupy an important place in this book. Similarly, the subject of "development" is broadly interpreted. Evidently, although this is not stated in the preface, Professor d'Amato's aim has been to describe those nuclear phenomena which are associated with the many aspects of development. A refreshing and often illuminating aspect of his treatment of the subject is the balance of examples from both the plant and animal kingdoms.

The first chapter deals with life-cycles, especially the alternation of diploid and haploid phases, and certain aspects of fertilisation. Chapter 2, on the cell cycle, opens with a brief excursion into molecular biology, discussing DNA sequence complexity, as well as the different classes of RNA. It is disappointing to find that a book published in 1977 should regard histones as "general repressors of gene activity", while making no mention of their important structural role in nucleosomes. This section seems rather oddly placed at the beginning of a chapter very much concerned with factors influencing the duration of different stages of the cell cycle, although also treating various aspects of chromosome replication. From the cell cycle, d'Amato turns to a well integrated account of meiosis, with special consideration of meiosis in relation to parthenogenesis.

It is not at all clear why chapter 4,

on "Mosaics and Chimaeras" was included. Although the subject is of great relevance to several problems of development, no special nuclear phenomena are described here, apart from a short mention of chromatin elimination in cases of gonadosomic mosaicism. This chapter highlights a problem which recurs to a varying extent throughout the book. There are many excellent and well described examples in every chapter, but insufficient attempt has been made to explain their relevance to the chapter in which they occur, or to the book as a whole. Since, as already noted, the author does not clearly state his aim in writing the book, it is particularly important that a common theme should be evident throughout the book. Such a theme eventually appears as one reads the book, but there are places where it seems that the author's enthusiasm for certain topics has led to considerable digressions.

The remaining half of this book sticks more closely to what seems to be its main theme: nuclear phenomena correlated with development. Two chapters deal with changes in nuclear DNA content during development: one with increases of the whole chromosome complement (essentially polyploidy and polyteny), and the other with differential DNA replication. There is much of interest in both these chapters, and the author's practice of taking examples from a wide variety of organisms is of particular advantage here. Evidently, DNA amplification, either selective or total, may be more important in differentiation than I. for one, had supposed.

Next come two chapters which cover what one might regard as the essence of a study of the nucleus in relation to development: gene expression and its regulation. It is axiomatic, as d'Amato clearly states, that differentiation must be viewed in terms of gene expression. Although none of the nuclear changes described earlier in this book can be causally related to differentiation, it is clear that single somatic nuclei of plants and (as described in the final chapter) of animals

contain all the genetic information necessary for normal development. d'Amato's consideration of gene expression concentrates on the localisation by *in situ* hybridisation of repeated genes, and on transcription in lampbrush and polytene chromosomes. These latter aspects could perhaps have been treated more extensively with advantage, for here are two types of chromosomes which afford unrivalled material for the study of nuclear cytology in relation to development. It is, moreover, a section which calls for half-tone illustrations, of which there are none in the book. If these were rejected on grounds of expense, it seems a false economy. (The book is nevertheless well illustrated by line drawings.) In the following chapters, the roles of heterochromatin, histones, and non-histone chromosomal proteins in gene regulation are considered, with the growing evidence for the importance of the latter being clearly brought out. The final chapter, "Regeneration and Totipotency," brings together information interesting in its own right, including the exciting experiments on nuclear transplantation in amphibia. This work, showing that nuclei of differentiated somatic cells still contain all the information necessary to produce a complete adult organism, is now such a cornerstone of developmental biology that it deserves an earlier and more prominent place in this book.

In conclusion, Professor d'Amato has brought together a wealth of information on the changes which occur in cell nuclei in different developmental situations. This is reflected in a bibliography of (the publisher tells us) over 1100 references, occupying nearly 50 pages. One wishes that the author had taken more space for general comment to integrate his material. Nevertheless, this is a book in which the specialist may well see new light shining on old problems, and the non-specialist read profitably for general information. • □

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## Electron spectroscopy

*Electron Spectroscopy: Theory, Techniques and Applications*. Vol. 1. Edited by C. R. Brundle and A. D. Baker. Pp. xv+459. (Academic: New York and London, 1977.) £24; \$46.90.

A MAJOR EFFORT to survey the present situation in electron spectroscopy has been undertaken by some of its more prominent explorers under the editorship of Drs C. R. Brundle and A. D. Baker. The material is distributed in three volumes, the first of which is now ready. To get a balanced impression of the total work one would have to await the other two volumes, since much basic and important material is still left to be treated in these forthcoming volumes. Already, after going through the first volume, one is, however, in a good position to see that this work will become an indispensable source of knowledge for all investigators in the field.

After an introduction to electron spectroscopy by the two editors, the present many-electron theory of photoelectron emission is reviewed by R. L. Martin and D. A. Shirley. The authors have themselves contributed a good deal to some of the conceptual parts of the theory for electron emission processes. After reading this and the next chapter by W. L. Jolly—treating his equivalent cores approximation—the reader should have obtained a good understanding of some of the main topics, which underlay most applications. W. C. Price, well known as an authority in 'classical' ultraviolet photon spectroscopy for molecules before photoelectron spectroscopy had appeared and also as one who has given distinct impetus to present-day photoelectron spectroscopy, treats the latter as applied to small molecules. Particularly interesting is his discussion as to why previous emission and absorption photon spectroscopy failed to reproduce the molecular electron level systems. He concludes his chapter thus: "In the brief period in which it has been developed, photoelectron spectroscopy has had phenomenal success in revealing the electronic structure of matter in a particularly direct way. Chemists can see the orbital structure of even fairly large molecules and no longer have to rely on the predictions of theoreticians. Future advances in the study of solids, adsorbed species, etc., can confidently be predicted to yield information on the nature and functions of the electrons involved. It is also clear that the subject will provide a happy hunting ground

for the physicist as well as the chemist for many years to come."

It is gratifying to see that indeed many chemists are already on the hunting ground and with most rewarding results. One of the great hunters is no doubt E. Heilbronner who, together with his colleague J. P. Maier in the next chapter, gives many illustrative examples from his rich scientific experience in the field of organic chemistry. A corresponding account from inorganic chemistry is amply given by R. L. DeKock, written, according to the editors' preface, during his stay in Beirut during "the troubled period of civil strife in Lebanon!"

High temperature studies of ultraviolet-excited electron spectra from

## World food supply problem

*World Food Resources: Actual and Potential*. By Michael Allaby. Pp.vii+418. (Applied Science: London, 1977.) £15.

THIS 'popular', readable, fact-packed book is presumably aimed at the 'informed layman'. But much of it should interest the scientist, agriculturalist or economist who wishes to see his speciality in better perspective. After an introductory chapter, comes a useful chapter (2) on population and on (economic as distinct from biological) food demand, followed by two chapters on regional nutritional status, the cost of 'modernising' farming, the 'green revolution', soils, water and fertilisers (3 and 4). Two historical chapters (5 and 6) are too UK-based to be useful. There then follow helpful chapters (7-9) about 'advanced' farming and its dependence on non-solar energy inputs, on its genetic vulnerability, on its ecological side-effects, and about alternative farm technologies.

An interesting chapter (10) on fish is followed by one (11) on land reform, rural employment, farm credit, and so on, and the *possible* (reviewer's italics) means of raising incomes and food production. Nevertheless, "we cannot be certain that those most in need of food will receive it" (p357). The final chapters (12 and 13) are politico-economic and examine the likelihood that effective international trade, aid and monetary devices would help to secure food for the under-fed, for example, by making phosphatic fertiliser available to poor countries.

Allaby, if not pessimistic, is doubtful about the likelihood of positive international action (chapter 13). But such doubts, one feels, are challenges to

halides is treated by one of the pioneers in this advanced form of the spectroscopy, J. Berkowitz, and the volume ends with a chapter on two-parameter coincidence experiments by M. E. Gellender and A. D. Baker. This technique, as pointed out by the authors, is more tedious than the usual electron spectroscopy but is instead applicable to, for example, ions of chosen excitation energies.

The applications of electron spectroscopy are expanding rapidly and already there is a problem to keep abreast with achievements in various fields. The present high quality volume is most helpful in this respect.

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those scientists and agriculturalists who are, for instance, seeking techniques to make available to crop plants the phosphate that is now immobilised in the soil, or to raise the lysine content of maize or sorghum, or to devise more productive farming systems (for example, the work at the International Institute of Tropical Agriculture, Ibadan, Nigeria). Allaby fails, it seems, to appreciate the scope for increasing, by relatively inexpensive means, the efficiency of human food chains. A major function of those trying to raise such efficiency should be, one feels, to make food production and consumption in poor countries as biologically independent as possible both of income gradients and of international squabbles, ideological differences, economic collapses and weather disasters.

The book as a whole lacks editorial discipline: chapter titles could be less 'slick' and more descriptive; more subtitles in the text would help the reader; not all the many tables are necessary although some of them, it must be said, are not available elsewhere in printed form. The references are inconsistently presented (some on tables, some at chapter ends, some in the Bibliography) and at least one is missing. Finally, many of the frequent acronyms are not defined or are enigmatic except to those who just happen to know, for example, that FAO/IWP means the Indicative World Food Plan (IWP) issued by the United Nations Food and Agricultural Organisation (FAO) in 1970.

This is a readable and useful, but sometimes diffuse, confusing and perhaps unduly pessimistic contribution on the world food supply problem. A much shorter, more disciplined version would be of greater use to the scientific community. A. N. Duckham

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## Evolution updated

*Evolution.* By T. Dobzhansky, F. J. Ayala, G. L. Stebbins and J. W. Valentine. Pp. 572 (Freeman: San Francisco and Reading, 1977.) £11.60.

EACH co-author has written four chapters in this book, setting forth his own ideas, and reflecting his own involvement with biology and genetics. I have a feeling that this has inevitably, although inadvertently, resulted in some overlap, not necessarily of illustrative material, but in the broad outlines of the ground covered. As might be expected, the most lucid passages are by Dobzhansky, to whose memory the book is dedicated. A notable 'one-liner' by him, "Nothing in biology makes sense except in the light of evolution", is on a flyleaf following the title page.

The chapter titles are as follows: "The Nature of Evolution", "Patterns of Speciation", "Evolution of Prokaryotes and Unicellular Eukaryotes", and "The Future of Evolution" by Stebbins; "The Genetic Structure of Populations", "The Origin of Hereditary Variation", "Phylogenies and Macromolecules", and "Philosophical Issues" by Ayala; "Natural Selection", "Populations, Races, Sub-species", "Species and Their Origins", and "Evolution of Mankind" by Dobzhansky; "Transspecific Evolution", "The Geological Record", "Cosmic Evolution and the Origin of Life", and "The Evolutionary History of Metazoa" by Valentine. These widely ranging topics certainly provide ample basis for a sweeping treatment of evolution.

Molecular matters have apparently been assigned to Ayala, who is a population geneticist. He also describes his studies of polymorphism in *Drosophila* populations of various localities. Valentine is a geologist and palaeobiologist, and he has provided an excellent discussion of the fossil record of evolutionary rates, and the record of ancient life and environment, including a welcome section on plate tectonics. Stebbins and Dobzhansky are both well known as authors of books and treatises on evolution.

In commenting on the 'evolutionary clock', Ayala discusses (p309) the comparison of amino acid differences between  $\alpha$ -haemoglobins of mammals and carp. He says that "the most recent ancestor to the four-legged mammals lived some 70 million years ago", and therefore he concludes that the comparison is useful for only 10% of the 700 million years of evolution of the  $\alpha$  chain. But this contention overlooks the fact that  $\alpha$ -haemoglobin sequences are also available for kangaroo, echidna, chicken,

viper and newt, and these fall in the intervening years. Ayala (p309) notes that differences between  $\alpha$ -haemoglobins of man and mice (17) are less than between rabbits and mice (28). (These differences are actually 16 and 27.) From this, he speculates that the rates of amino acid replacement may be increased by shorter generation time. The data he cites, however, are too scanty. If the  $\beta$  chains are compared, the man-mouse difference is 28 and the rabbit-mouse difference is 29.

The subject of evolution embraces both the past and future of human beings. It therefore presents an irresistible temptation to indulge in philosophical discourse. Three of the authors have availed themselves of this opportunity, and Ayala holds forth on "Philosophical Issues" for the final 42 pages, in which he provides us with such insights as "Common sense tells one that children resemble their parents and that good seeds produce good crops", and "No organism can be truly independent of the environment". I felt that Stebbins, and, more especially, Dobzhansky, had provided sufficient (and excellent) treatment of dissertational matters in chapters 14 and 15, so that the last chapter was not needed.

In a comparison of cytochrome sequences, the table on pages 296 and 297 shows only 20 identical positions shaded in grey, but 36 positions should have been so indicated. In addition, the

sequence of residues 27-34 in *Neurospora* cytochrome is erroneous.

Figure 2-3, page 26, depicts the anticodors for UUU and GGU as AAA and ACC. These should be GAA and GCC. Figure 3-1 shows an erroneous codon for methionine, and represents purines by "Y", which is the symbol for pyrimidines.

All in all, the book is an excellent and up-to-date treatment of evolution, the subject that has spread like a network to encompass all the biological sciences. I could not help contrasting the wealth of material in this book with the fact that creationists are still largely successful in their efforts to minimise the teaching of evolution in schools in the USA. In June 1977, a member of the California State Board of Education was able to expunge or garble certain sentences descriptive of evolution in the forthcoming new edition of *Science Framework*, a booklet of guidelines for teaching science. For, as Dobzhansky says (p439): "The mutually sustaining effects of biologists, paleontologists and anthropologists have made the theory of the evolutionary descent (or rather, ascent) of man impregnable . . . And yet some antievolutionists persist. Some of them are simply ignorant of the evidence, while others have so prejudged the question that no evidence is meaningful to them".

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## Botanical heritage

*Dictionary of British and Irish Botanists and Horticulturists: Including Plant Collectors and Botanical Artists.* By Ray Desmond. Pp. xxvi+747. (Taylor and Francis: London, 1977.) £40.

If there is one area in which the British Isles has been highly productive, it is in the generation of botanists, yet few of their names have survived except possibly in the specific names of certain obscure plants. Names like Ray, Gerard, Turner and Culpeper may still be familiar to many, especially those caught up in the recent general enthusiasm for health foods, herbs, and so on. But many more names have now passed into oblivion as botanical exploration and taxonomy have progressed. This *Dictionary* has grown out of a desire to catalogue our British and Irish botanical heritage and to collate what information can be recovered concerning the achievements of our deceased, plant-hunting forefathers.

Each entry is set out in the form of details of date and place of birth and death, education, qualification and

selected publications. Information concerning biographies, obituaries, herbaria and species graced with the botanist's name are also given in abbreviated form. Only dead botanists are included, but all spheres of the subject are covered, from nurserymen to palaeobotanists and physiologists.

The obvious question one must ask when faced with this hefty and expensive tome, concerns the use to which the book may be put. It is not a collection of historical anecdotes, and will not, therefore, serve as a book into which one may dip for entertainment. Its value is likely to be appreciated only by those undertaking serious historical studies into the development of botany in Britain. It is a book which will thus find its way into specialist libraries, but at this price it is hardly likely to attract the attention of individual botanists. It is essentially a work of reference which will lead the historically minded to obscure sources which would not otherwise be easily traced.

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## Neutron scattering

*Neutron Scattering in Chemistry.* By G. E. Bacon. Pp. 186. (Butterworth: London and Boston, Massachusetts, 1977.) £12.50.

IN writing this book, Professor Bacon has attempted a very difficult task. It is difficult firstly because any technique is likely to be able to contribute to many different branches of chemistry and secondly because neutron scattering is several techniques rolled into one. On the one hand, it is a structural tool and so immediately embraces the whole range of structural work on condensed matter including crystallography. In addition, however, the magnetic dipole moment of the neutron makes possible the study of magnetic structures and hence the determination of unpaired spin densities. On the other hand, it is also a spectroscopic technique because the low energy of neutrons of wavelength comparable with molecular dimensions makes possible an energy analysis of scattered neutrons so as to reveal details of atomic and molecular translational, rotational and vibrational motions. It is this unique ability to probe explicitly the time-dependent structure that makes neutron scattering so powerful. The range of problems which can usefully be studied, however, is always limited by the available neutron fluxes.

For many years, it has been possible to study relatively complex structures but when the fourth dimension of energy analysis is added the flux determines the practicable energy resolution and therefore the complexity of the problem which is amenable to study. A major advance in this respect was provided by the advent of the latest generation of high flux reactors within the past few years, and in particular the unique instruments at the Institut Laue Langevin, Grenoble. It is unfortunate that the timing of this book is such that only a very little of this exciting new work could be included.

Professor Bacon (a Professor of Physics) has attempted to give a brief (~ 180 pages) and essentially qualitative description of some of the results of applying neutron scattering techniques to various areas of chemical interest. It is a book in which to find a summary of some of the things which have been or can be done using neutrons, rather than a detailed description of the underlying theory or of the chemistry. For the reader who wishes to pursue a topic to a greater depth, there is a general bibliography of neutron scattering texts and conference proceedings and a list of references with each chapter.

The first two chapters contain good, succinct descriptions, respectively, of the

Principles of Neutron Scattering and Experimental Methods. Three chapters follow on crystallographic studies (Structural Studies, Direct Methods, and Correlation of X-Ray and Neutron Data: X-N Syntheses) which contain some well-chosen examples of recent work. A brief chapter on Studies of Biological Materials contains a timely discussion of small angle scattering methods, but it is surprising to find no mention of specifically chemical applications of these techniques, such as the important studies of polymeric materials. Two further chapters complete the discussion of crystal structure investigations; the first (Measurements of Covalency) is concerned with magnetic scattering and the second with both Bragg reflection and diffuse scattering studies of Defects and Non-Stoichiometry in a variety of crystalline phases.

In all of the above, as well as in the structural aspects considered in the last chapter on Liquids, Glasses and Gases the treatment is clear and authoritative. The two chapters not so far mentioned are entitled Molecular Spectroscopy and

Polymers. Here, the treatment is less satisfactory partly because, as mentioned earlier, it is in these areas where marked advances have very recently been made, since the availability of high resolution spectrometers. Nevertheless, this does not account for all the omissions: for example, there is no discussion of quasielastic scattering from molecular rotations, nor of solid-state diffusion; indeed, the treatment of quasielastic scattering does not begin to reflect the importance of this area of work (the omission of any reference to Springer's elegant 1972 monograph on the subject demonstrates the point). There should have been some mention of phase transitions and a more extensive discussion of phonon dispersion in molecular crystals. In spite of the omissions, however, this is a timely and welcome book which goes some way towards filling a serious need

A. J. Leadbetter

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## Raman spectroscopy

*Raman Spectroscopy.* By D. A. Long. Pp. xiii+276. (McGraw-Hill: New York and London, 1977.) £14.40.

FOLLOWING the development of the laser in the early 1960s, there has been a boom in Raman spectroscopy. It is now a routine laboratory technique. This rapid progress has led to the publication of several multi-author volumes and to the *Journal of Raman Spectroscopy*. This book, however, is the first by a single author that aims to provide an "up-to-date survey of the whole subject, setting Raman spectroscopy in perspective, unifying the basic theory, illustrating the applications and potential of the technique, and guiding the reader towards the specialist literature".

There are eight chapters and three appendices. Also, there is a central reference section of sixteen blue pages containing thirteen tables of formulae for intensities and polarisation properties of Raman scattering by gases. Chapter 1 is a general introduction, a nice feature being the reproduction of the original 1928 papers by C. V. Raman and K. S. Krishnan and by G. Landsberg and L. Mandelstam in *Nature* and *Naturwissenschaften*, respectively. Chapter 2 describes the properties of electromagnetic radiation and defines the four Stokes parameters. Chapters 3 and 4 present classical and partial quantum-mechanical treatments of Rayleigh and Raman scattering. They proceed at an easy pace and include a very detailed account of the

polarisability tensor. Chapter 5 is on time-dependent perturbation theory, the Placzek polarisability theory, resonance Raman scattering, and Raman optical activity. Experimental procedures are described in chapter 6 and numerous examples are discussed in chapter 7. The final chapter is devoted to non-linear Raman effects. Appendix I is a guide to the literature; II lists for the common point groups the symmetry classes of translations, rotations, polarisabilities and first hyperpolarisabilities; and III describes a Raman study of a crystal.

The style is lucid and the illustrations excellent (although the notation is not always attractive), so the book will be helpful to many students. It expounds at length some of the properties of cartesian tensors, and includes some very interesting and recent applications of Raman spectroscopy to problems in organic and inorganic chemistry. Some of the more physical topics, such as line shapes and Brillouin scattering from liquids, are not considered. In a few places, as in the description of magnetic and electric Raman optical activity on p131, there are errors; and the references are not always adequate (for example, the four references at the end of chapter 3 and those in Appendix I give no help to someone trying to understand non-linear polarisation, introduced on p41).

The book has been well produced and is good value.

A. D. Buckingham

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# obituary

## Gersh Budker

PROFESSOR Gersh Itskovich Budker, Director of the Institute of Nuclear Physics of the Siberian Branch of the Soviet Academy of Sciences, died on 5 July 1977. With his death we have lost one of the most imaginative and colourful accelerator scientists.

Professor Budker was born on 1 May 1918 in the Ukraine. He graduated from Moscow University in 1941 and, after having served in the Soviet Army until 1945, began work at the Institute of Atomic Energy of the USSR Academy of Sciences. He was initially involved in nuclear reactor development but his interests gradually moved to accelerators for elementary particle research. In 1956 he also became professor at the Moscow Engineering Physics Institute.

In the same year the Scientific Research and Educational Centre, Akademgorodok, was created just outside Novosibirsk. This was a great challenge to creative young scientists, and it was natural that Budker, in 1957, was appointed director of its Institute of Nuclear Physics. This Institute has been the source of an almost continuous stream of ideas and novel accelerator systems and very soon became known throughout the world.

In his new position Budker was able to realise some of the plans he had already developed while at the Kurchatov Institute in Moscow. His main theme was to provide colliding-beam facilities so as to bring his institute to the forefront of elementary particle physics without the heavy expenses required for more conventional accelerators. He was not only afraid of great expense (he even sold accelerators in the 'open' market to boost his budgets), but also afraid of the inflexibility inherent in very large installations. The programme he established was ambitious and daring and made heavy demands on ingenuity and drive.

He had his first storage ring, VEP-1, working in 1963. An electron-positron device, VEPP-2 of  $2 \times 700$  MeV, was in operation from 1967, another one, VEPP-3 of  $2 \times 2.2$  GeV, in 1972, and a fourth one, VEPP-4 of  $2 \times 7$  GeV, is at present under construction. They have made it possible for his institute to



Camera Press

perform front-line experiments on meson resonances. Some of the rings have also been used as a source of synchrotron radiation for research in solid state physics, etc.

Budker had incorporated a two-way proton colliding beam device in the original plans for his Institute. He modified this in 1966 when he invented the so-called electron cooling of proton or antiproton beams, which made it feasible to propose instead a colliding beam project with antiprotons against protons. The electron cooling technique was essential for the accumulation of sufficiently intense beams of antiprotons.

But first the cooling principle had to be verified experimentally. With the limited means available at the institute this took longer than Budker had hoped and delayed the proton-antiproton plans. However, in 1974 his institute could report most beautiful experimental evidence for electron cooling of a proton beam. This invention and its experimental verification must be considered the peak of Budker's career. The technique and its possible uses are being discussed in accelerator laboratories all over the world, and it is being considered in important plans both at the European Organisation for Nuclear Research, CERN, and at the Fermi National Accelerator Laboratory in the United States.

Although Budker and his institute became best known because of the work on accelerator devices, the institute also has a branch that carries out research in plasma physics, which gives a very fruitful cross-fertilization between plasma physics and accelerator physics.

Professor Budker distinguished himself not only as a scientist, but also through his exceptional ability to stimulate and inspire his collaborators. His Institute is now 20 years old and has many remarkable achievements behind it. Nevertheless, it still retains an unusual pioneering spirit. Budker also felt very deeply about the whole philosophy that lay behind Akademgorodok and was a driving force in ensuring that this unique place became a centre of scientific achievements with a world-wide reputation.

Budker was a member of the USSR Academy of Sciences. In 1967 he received the Lenin Prize for his contributions to the development of colliding beam devices.

K. Johnsen

## Milton N. Bramlette

MILTON NUNN BRAMLETTE, the distinguished American geologist, died on 31 March, 1977. He was born in Bonham, Texas, on 8 February, 1896, attended preparatory school in St. Louis, and entered the University of Wisconsin, Madison, in 1914.

His university studies were interrupted by World War 1. He enlisted in the aviation service, and qualified as a pilot, but too late in the war for combat service. He was discharged early in 1919 as a 2nd Lieutenant, and returned to Madison, where he graduated A.B. in 1921. During two summers he had served as assistant on the Wisconsin Geological Survey, running magnetic traverses across the iron ranges of northern Wisconsin.

With this field experience and high academic record, he was appointed Assistant Geologist in the U.S. Geological Survey in 1921. While headquartered in Washington he commuted to Johns Hopkins for advanced work in stratigraphy. After field mapping in Montana, Kansas and South Dakota, he took leave of the Survey



for graduate work at Yale. This was interrupted by field work in Mexico and Venezuela for the Gulf Oil Company. He returned to Yale for further graduate studies in 1928–1930, when he resumed his appointment with the Geological Survey. His doctorate was received *in absentia* in 1936.

Bramlette soon entered into a close and remarkably fruitful association with that outstanding specialist in Mollusca, Wendell P. Woodring, for a series of brilliant stratigraphic studies in California: the Palos Verdes Hills, Kettleman Hills and Santa Maria Valley. It was then that Bramlette began his petrological studies of the sedimentary rocks, dealing especially with intrastratal alterations, phosphates, zeolites and siliceous cements, culminating in his classic paper on the cherts of the Monterey Formation. He also began his studies of Cenozoic micropalaeontology and with his keen discernment was soon able to identify many of the diagnostic foraminifera in the field with a hand lens.

Bramlette was a principal investigator, along with W. H. Bradley and K. E. Lohman, of the transatlantic series of Piggott cores during the 1930's. With our current plethora of much longer deep sea cores, this work has been nearly forgotten; nevertheless it was the first study of deep-sea stratigraphy and was highly significant in permitting the first correlation of European and American glaciations. At this time he laid the groundwork, through his studies of the Arkansas bauxite deposits, for the great increase in productivity of these mines during the submarine-induced crisis of the second world war.

In 1940 Bramlette joined the faculty of the University of California at Los Angeles, where he remained until his transfer to the Scripps Institution of Oceanography in 1951, except for two years during World War II, when he worked on the bauxite deposits of Arkansas and Jamaica.

At the Scripps Institution Bramlette began his highly rewarding studies of the fossil coccoliths. Because of their minute size and irregular forms, these organisms sink very slowly in the sea so that currents distribute them almost world-wide. Accordingly they constitute unusually valuable time markers for the widespread correlations of marine sediments. Bramlette's contribution to the development of such correlations was pre-eminent, and he continued as a world leader long after his formal retirement in 1964.

Bramlette's versatility and profound scholarship were outstanding. He was elected to the National Academy of Sciences in 1954, was awarded its Thompson Medal in 1964, the Distinguished Service Medal of the Depart-

ment of the Interior in 1963, and the Doctor of Laws degree from the University of California in 1965.

He died of emphysema on March 31, 1977. He was a modest gentleman.

James Gilluly

## A. L. Walpole

DR ARTHUR WALPOLE died at Wilmslow, Cheshire on 2 July 1977, aged 64. Trained initially as a chemist (Imperial College London, B.Sc., Ph.D.), he spent a short period in the Department of Pharmacology, Edinburgh, and then joined the biological section of the new pioneer group in the Dyestuffs Division of ICI Ltd, in Manchester, which ultimately became the Pharmaceuticals Division.

His early interest, on the implication of novel synthetic oestrogens in the treatment of tumours, coupled with the appointment of Professor (later Sir) Alexander Haddow as a consultant, began Walpole's concern with cancer research, for which he is best known. His work fell under two headings. First came his collaboration with Michael Williams, who as Medical Officer for the Dyestuffs Division was deeply involved in its associated industrial hazards. Together over the years, with *ad hoc* synthesis where needed, they worked out structure/carcinogenic relationships, particularly amongst the arylamines, which still remain of world-wide significance.

Alongside this, and arising in part from the textile interests of the Division, Walpole was amongst the earliest (1947 onwards) to study the effect of introducing alkylating groups such as methylolamido, epoxide and aziridyl into potential tumour-inhibitory structures. His recognition of the peculiar cytotoxic properties induced by these moieties again had implications for industrial safety.

Walpole's other over-riding interest was in the hormonal control of reproduction and related phenomena. Thus researches around basic derivatives of the oestrogenic triphenylethylenes have recently led to a compound especially useful in the treatment of certain forms of mammary carcinoma. Likewise, he pioneered parallel studies in animal husbandry, first using certain disthioureas, and later the new synthetic prostaglandins. He was engaged on this latter work at the time of his death, two years after formal retirement.

As a person, he naturally attracted a wide circle of friends and collaborators, and his other qualities extended deeply

into the arts. Above all, he was much concerned for humanity, whether in the mass or the individual. He leaves a widow, Dora.

F. L. Rose

## G. G. Villela

PROFESSOR Gilberto G. Villela, the founder of modern biochemistry in Brazil, died on 17 July 1977, aged 73.

Dr Villela was born in Minas Gerais, Brazil, on 12 July 1904. After graduating in medicine in 1926 he entered the Oswaldo Cruz Institute with which he was associated, first as a student and then as a member of the staff, until the time of his death.

Dr Villela was an authority in the field of biochemistry and he made many significant contributions specially in vitaminology and enzymology. Another field of active interest was the biochromes, where he made many contributions to the study of animal pigments. His publications, more than 300 papers, appeared in medical and biochemical journals in Brazil, Britain, the United States and elsewhere. He was also the author of several scientific books.

Dr Villela visited, lectured and carried out scientific work at many important research centres all over the world, including the University of California Medical School (1945), University College London (1949), the Pasteur Institute (1950), and New York University (1961), where he was a Visiting Professor. As a UNESCO representative he set up a new centre of biological research at the University of Rangoon, Burma (1967). More recently he went to Japan as a lecturer, at the University of Nagoya (1973) and as Chairman of the Xth International Congress of Nutrition in Kyoto (1975).

Dr Villela was elected to several international scientific societies and received several medals and prizes. He was the founder and first President of the Brazilian Society of Biochemistry, and attended most of the Society's meetings.

Dr Villela's life was fully devoted to his scientific interests which he pursued with enthusiasm and vigour. To his co-workers and students he was an inspiring and ever-helpful teacher. He enjoyed discussions; his mind was very critical but combined with a good sense of humour. He was a man of high moral standing and humanistic education. He will always be warmly remembered by all who knew him.

He is survived by his widow Regina, one son, Gilberto, one married daughter, Sonia and a grandson, Pedro.

Emilio Mitidieri

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## Don't squander precious time on genes

THE House of Commons Select Committee on Science and Technology is one of Britain's more valuable Parliamentary institutions. In the past it has held a number of important and useful inquiries—into Lord Rothschild's proposals for reorganising research council funding, for example, and Britain's choice of nuclear reactors. Even if the conclusions of such inquiries have been open to criticism, they have often thrown much-needed light on some of the murkier corners of government decision-making.

It is, however, difficult to be entirely enthusiastic about the select committee's decision last week to establish a sub-committee on genetic engineering—which presumably means the implications of current research using recombinant DNA techniques. Not that the subject lacks widespread public concern (perhaps more so in the United States than in Britain), nor that it is one on which politicians, in whose lap some of the important decisions may ultimately fall, are well informed. But the timing is wrong.

It is now over three years since the moratorium on recombinant DNA was announced by a group of scientists in the US, and in that time much has happened. Every country which carries out such experiments has now developed some form of control mechanism (some, admittedly, more effective than others). And in the process, the issues

raised by the experiments have been subject to exhaustive scrutiny and debate. As a result, there now seems to be a general feeling that initial reactions may have been too strong. This cooling-off has undoubtedly been partly due to the successful lobbying of those with scientific and other interests in the continuation of such experiments. But there is also a general feeling among many scientists that the dangers, although real and potentially extremely hazardous, have been overplayed.

In these circumstances, it is difficult to see what the select committee will be able to achieve. The area is not one which, like some previous issues, can claim to have suffered from lack of public debate. This is not to claim any particular immunity for decision-making processes within the scientific community. But although such an inquiry would have been useful two years ago, there seem to be more pressing and potentially significant issues at the present time. Examples might be the future needs for scientific manpower, or the definition and management of technological risk in general (which might, indeed, still be embraced by expanding the terms of reference of the new subcommittee). The select committee's time is a precious commodity; it would be a pity if it was squandered on re-opening the scars of previous battles. □

## China's path ahead

WE bring together in this issue of *Nature* a number of reports on recent developments in science within the People's Republic of China, together with a note on the Peking/Taipei conflict, which is at present reverberating within the international unions. Some of the barriers between the People's Republic and the rest of the world were lifted five years ago. Yet it is still possible for the Chinese to spring surprises on us—with their great wealth of data, their respect for the role of the amateur in some scientific fields, and most recently with the obvious signs of a profound interest in re-establishing science and technology as a thoroughly professional pursuit. This latter trend can be seen not only in the way that university and institutional research is being revived for the purpose of obtaining economic benefits for the nation, but also in the way that certain selected branches of science with less obvious practical benefits are to be stimulated. The all-China meeting in the Spring to discuss national policies on science and technology is bound to be a fascinating occasion.

The path ahead for China is by no means simple, however. Renewing a respect for pure research, for theoretical

understanding as well as practical achievement, even for an elitist rather than populist approach, will not be easy after half a generation in which many of the invisible threads of tradition in the scientific method may have been damaged. But perhaps an even more difficult task will be for the Chinese to hold fast to those elements of people's science which have proved so successful in recent years, most notably the accurate reporting of natural phenomena whether they be connected with health, agriculture or stirrings within the earth. For in some instances this mobilisation of the masses has had major successes—the use of amateur scientists in earthquake prediction, for instance, with many quakes now successfully predicted, puts the Chinese well ahead of the rest of the world.

How maintain a broad public involvement in science and technology while giving the experts their head? And how cope with an almost inevitable demand from a newly-arisen intelligentsia for a greater extension of human rights and liberties? These are among the questions that China must confront in the next decade with its new mood of enthusiasm for science. □



# Science in Hua's China

## The key to modernisation

Science is undergoing a renaissance in post-Mao China. The United States National Academy of Sciences has been keeping track of the changes, as **Mary Brown Bullock** reports



IN recent months many visitors to China, and the Chinese press, have been recording a dramatic change in China's science and technology policy. Since the "smashing of the gang of four", China has embarked on a pragmatic drive to achieve the "four modernisations"—industry, agriculture, national defence, and science and technology—by the year 2000. The significant aspect of the current use of this slogan is the new emphasis being placed on science and technology as, in Chinese parlance, the "key link" in supporting the other three sectors.

The scientific renewal has been explicitly endorsed in speeches by Chairman Hua Kuo-feng and Vice-Premier Teng Hsiao-p'ing. It has also been evident in a spate of Chinese newspaper articles, scientific conferences and meetings, with the promise of more to come. As a result the broad outline of China's revitalised science policy have become evident.

The new focus includes basic re-

search, selective learning from foreign sources, specialised graduate training, and a new respect for professionalism. In the terms of China's 25-year-old science policy debate, the tension between "reds" and "experts" has eased. Both the masses and the professionals have contributions to make, but the cutting edge has returned to the experts.

Some of these trends were observed by a high-level delegation of American scientists led by Dr Philip Handler, President of the National Academy of Sciences, and sponsored by the Committee on Scholarly Communication with the People's Republic of China, which visited China in June. During visits to five universities and more than 25 research institutes in Peking, Tsingtao, Wuhan, Shanghai, and Nanking, delegation members observed new vitality in research institutes, planning for more specialised graduate education, and a greatly strengthened role for the Chinese Academy of Sciences.

These early impressionistic observations have been confirmed during the past four months by the political reinstatement of China's patron of science and technology, Teng Hsiao-p'ing, by official policies promulgated by the 11th Party Congress, and by numerous articles in the Chinese press. Furthermore, the selection as a member of the Politburo of Fang I, Vice-President of the Chinese Academy of Sciences, has continued to enhance the position of the Academy.

A ministry-ranked institution, the Academy is now being assigned a major role in providing the scientific and technical expertise necessary to modernise agriculture, industry, and national defence. The Academy's research institutes, of which there are over 60, and which were formerly under attack for their 'elitism', now receive encouragement and support from central and local authorities.

### New policies

Two articles by leading Chinese scientists published in the authoritative, and usually political, *Red Flag*, reflect a number of the new policies. The first, entitled 'Science and technology must catch up with and surpass advanced world levels before the end of the century', was written by Chien Hsueh-sen, a former aerodynamics and rocket expert at California Institute of Technology, who returned to China in 1955. Published in early July as one of the first detailed discussions of China's new science policy, the emergence of the western-trained Chien as official spokesman itself symbolised a major change.

In a rather candid portrayal, Chien acknowledged a disparity between the state of Chinese and world science. But he went on to affirm that China could catch up through selective, critical



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learning from foreign sources, provided that "the role of the professional contingent in the field of science and technology" is brought into full play. He lists four basic areas for improvement: data compilation, scientific instruments, quantification and standardisation, and scientific and technical publications.

The title of the second article, 'Bear in mind Chairman Mao's concern for and teaching on education in science and technology', is almost self-explanatory. Written by Chou P'ei-yuan, vice-chairman of the Chinese Scientific and Technical Association, it cites Mao Tse-tung repeatedly in affirming the importance of theoretical research in the natural sciences and the need to up-grade China's major universities. Chou joins a host of other authors in invoking not only Mao, but more convincingly former Premier Chou En-lai, in legitimising this renewed emphasis upon science and technology.

### Science conference planned

Political support for Chinese science is also provided by China's present-day leadership. Chairman Hua has personally addressed a number of scientific conferences, and has called for a national conference on science and technology to be held next spring. But it is Vice-Premier Teng Hsiao-p'ing's imprimatur which is most evident.

One of the key signals that a new policy was in effect, and that Teng was about to be rehabilitated, was the official praising of Teng's 1975 'Outline report on the work of the Academy of Sciences'. Repudiated when written as a "poisonous weed" by the 'gang of four', this article urged raising research standards within the Academy's research institutes.

Not only are Teng's pragmatic concepts now flourishing, but he also appears to be following Chou En-lai's footsteps by taking a personal interest in meeting visiting American scientists, accompanied by senior Chinese scientists. These meetings, announced in the Chinese press, play an important part in enhancing the role of China's intellectuals.

It is too early to identify specific new directions in Chinese scientific and technological research. The conferences which have already been convened at both the national and provincial levels have been on a range of topics, including high energy physics, agricultural sciences, and geology. Seminars have been held on developments abroad in electronics, laser technology, and communications, fields which have featured prominently in international exchanges. As more details unfold concerning a proposed 1978 National Conference on Science and Technology, and as publications ex-

pand, additional research priorities should become evident.

It is also too early to predict how modifying China's former autarky will affect its international scientific and technical relations. It is important to realise that modest exchange between Chinese and Western scientists has been underway throughout the 1970s. For example, over thirty Chinese scientific delegations have visited the United States since 1972 under the centralised auspices of the Committee on Scholarly Communication with the People's Republic of China. Many more scientists and engineers have participated in commercially-related programmes.

Other nations have had similar experiences, and also have begun longer-term individual visits. Significant signs of a changing Chinese posture might be expected to include an increase in Chinese scholars studying abroad, the inauguration of cooperative scientific research programmes, and expanded purchases of foreign technology.

There has recently been an expansion in the numbers of scientists and engineers from all countries visiting China, and they describe a scientific renaissance. Individual Chinese scientists describe themselves as being "jubilant" over the new policies. Hosts have been eager to arrange substantive itineraries and to provide opportunities for foreign scientists to meet individually or in small discussion groups with Chinese colleagues.

During October, for example, a series of joint seminars on specific topics in cancer and astronomy are being arranged for two CSCPRC delegations. Americans hosting visiting Chinese scientists and engineers have likewise remarked on the relaxed and

candid attitude of their visitors. A new dialogue appears underway.

It is perhaps significant that leading Chinese scientists, Chou P'ei-yuan and Pei Shih-chang, were seated at the head table with US Secretary Cyrus Vance and the Chinese Foreign Minister, Huang Hua, during Vance's August trip to Peking. However, since the absence of diplomatic relations may provide some restrictions to programmes with the United States, China's interchange with Britain, Western Europe, Japan, and Australia will probably provide the first signs of major policy change. In this connection, an exchange of high level groups between the Australians and the Chinese Academy of Sciences has recently been planning seriously for longer-term programmes.

Since the beginning of this decade, the developing and industrial world has been fascinated with China's attempts to fashion a science and technology policy which includes the masses, traditional science, and indigenous technology. One need only remember the eruption of interest in the early 1970s when the rest of the world 'discovered' acupuncture and barefoot doctors.

World attention is now focusing on the more technical, professional aspects of China's new policies. Major changes in science and technology policy are certainly underway. But this new emphasis will not entirely replace the heritage of the Cultural Revolution. Chairman Mao's dictum that science should "serve the people" has been refined by Chairman Hua's "May science and technology flourish, and may the good news pour in". The latter part of that phrase, "good news pour in", however, suggests that results, now as before, are still expected. □



Chinese seismologist briefs American delegation on Hsinfeng dam, near Canton



# Mass surveys to detect cancer

**Eleanor Lawrence**, who talked to an American scientist recently returned from China, reports on the particular interest China holds for the cancer epidemiologist

A GROUP of cancer specialists was among the first medical teams from the United States to visit China in the more relaxed atmosphere following the fall from power of 'the gang of four'. And according to one of the team, Dr Robert W. Miller, Chief of the Clinical Epidemiology Branch of the US National Cancer Institute, China turned out to be an aetiological wonderland for the cancer epidemiologist. In particular, the causes of several cancers, unusually common in certain regions of China are becoming amenable to study. The clues come from parallel incidences of similar cancers in domestic animals in the epidemic areas. The parallels have come to light over the past decade as a result of mass surveys. The patterns of incidence strongly suggest environmental causes for these cancers and this is the aspect the Chinese appear to be tackling most vigorously.

Mass surveys and screening, largely carried out by the barefoot doctors and local people, are the most important and most emphasised aspect of China's fight against cancer. The emphasis is on identifying high risk areas, early diagnosis and treatment, and prevention by health education. Where early diagnosis coupled with simple surgery is likely to be successful, as for oesophageal cancer, it is widely practised in the country hospitals and the success rate is high. Radiotherapy and sophisticated chemotherapy is available in hospitals in the large cities but Dr Wu Huanhsing, head of the Oncological Hospital of the Chinese Academy of Sciences in Peking believes that "you can't treat cancer with a big machine".

Dr Li Ping, Deputy Director of the Cancer Research Institute in Peking, showed the American team the first results of a massive survey of cancer mortality, which eventually will cover all of China. Deaths from the commoner types of cancer are recorded commune by commune (each commune consists of about 8,000-10,000 people) and maps made of the relative incidence of each type of cancer within a province. Of the 29 provinces in China, one has already been completely mapped and six more are in preparation. Already sharply-defined areas of high incidence of liver cancer, for

example in southern China, can be seen, whereas lung cancer deaths are distributed non-randomly (as in similar maps prepared in the United States by the National Cancer Institute). Writing in the English language magazine *China Reconstructs* (October 1977) Dr Li estimates that a million barefoot doctors are engaged in the survey.

## Mapping high incidence areas

The Chinese have set up some 30 bases for cancer study, prevention and treatment in villages, factories and mining areas throughout China, in areas with high incidences of cancer. The clearest picture so far of cause and effect has been obtained for oesophageal cancer, which has an unusually high incidence in some regions of northern China, focused on Linhsien county. Among the 700,000 people in the county one new case of oesophageal cancer is diagnosed every 8 hours, some 50 times the rate for US whites.

The high incidence of human oesophageal cancer is paralleled by a similar excess of gullet cancer in domestic chickens in the area, leading to the suspicion that both cancers might have a similar environmental cause. Important clues to the possible cause have been uncovered by the Chinese following the relocation of more than 50,000 people from Linhsien county to Changshun county, 350 miles away, where the incidence of oesophageal cancer is low. When the people moved, in 1967-68, to allow a new reservoir to be built, they apparently did not take any of their

chickens with them but bought new birds when they arrived in Chungshan, from flocks hitherto free of gullet cancer. Since then, however, 12 chickens of the 5,500 owned by the immigrants have developed gullet cancer although no cases have been reported in about 2,400 chickens belonging to the native population. This pointed to a carcinogen limited to the chicken's immediate environment, but not apparently confined to Linhsien county. One suspect may be a pickled vegetable mix, a speciality of Linhsien, which could also be fed to the chickens in table scraps. Nitrosamines have been found in this mix and the American team has brought back samples, which will be tested in the Ames test amongst others, for mutagens which may also be carcinogenic.

The American team commented that changing the chicken food from table scraps to grain would provide an unambiguous answer to whether the carcinogen was in food eaten by both chickens and people. The Chinese are indeed mounting a campaign in Linhsien county itself which is aimed at: preventing food and grain from moulding (toxins produced by some mould contaminants are suspected carcinogens), correcting a molybdenum deficiency in the soil, removing excess nitrates and nitrites from water and "changing undesirable dietary habits" —presumably to eliminate food containing nitrosamines. They are also now able to detect oesophageal cancer in its earliest stages by mass screening and the cure rate, by surgery, is apparently very good.

## Pinpointing several causes

According to Dr Miller the Chinese are not so interested in pinpointing a specific cause, as in preventing cancer by controlling a range of possible causes. They believe that there is no one specific cause for any type of cancer and that at least four factors will be involved. This perhaps accounts in part for the resistance the American team found to the idea that lung cancer is overwhelmingly caused by cigarette smoking. A substantial proportion of the population in China now smokes and the lung cancer rate is also increasing, although because cigarette smoking only became widespread in China some 20 years ago the full impact has not yet been felt.

Another cancer which has an unusual incidence in China and which also seems to be associated with a similar cancer in domestic animals is nasopharyngeal carcinoma, which seems to cluster in southern China. In a rural area 100 miles from Canton, the American team talked to a barefoot doctor who told them that six people



Storage jars for clean, uncontaminated grain.

This visit was sponsored by the National Academy of Sciences Committee on Scholarly Communication with the People's Republic of China.



in the 3,000 under his care had developed the tumour in the past decade. Only when they returned to Canton did the American scientists find that nasopharyngeal cancer had been found to cluster in pigs elsewhere in Kwangtung Province—the 'snuffling pig' syndrome. As with the chickens, this could point to a carcinogen limited to the immediate environment of the pig, a much more restricted environment than that of humans and easier to

analyse.

Hepatocellular cancer (liver cancer) is 'epidemic' throughout southern China and the Chinese are looking at hepatocellular cancer and cirrhosis in domestic ducks, although no systematic survey has apparently been made. Studies on the inherited susceptibility to various types of cancer are not apparently given great prominence in China, prevention and early detection and treatment being stressed. But the

American team obtained anecdotal evidence of several instances of very strong familial clustering possibly indicating genetic susceptibility.

The American team also included pathologists, pharmacologists and clinicians and a full report of the visit will be published next year by the National Academy of Sciences. In the present climate of a free exchange of information, it should be one of the largest reports yet. □

## Two major controversies

**T. B. Tang** traces recent changes in China's education policy and the status of her scientists

**F**OLLOWING the death of Chairman Mao and before the 'gang of four' fell from power, the two major controversies in China concerned cultural activities and the educational system. It was in these spheres that the 'gang' exerted influence for the longest period of time, and it is therefore not surprising that these fields are now seeing the largest and most definite changes, with important implications for the development of science and technology.

One early indication of this was the widely-publicised memorial meeting held for Chou Jung-hsin in August. Chou, who died last year, was a former Minister of Education, and had previously held a leading post in the Academia Sinica; it was disclosed that he had been "framed" by the 'gang', and had suffered considerably as a result. An article by the Ministry of Education in the current issue of *Red Flag* now asserts that the educational policies in force during the 17 years before the Cultural Revolution were basically correct. It implies that the "Sixty-Point Document on Higher Education" prepared by the Ministry in 1961, could still be relevant today.

Recent events indicate increased concern over higher education in science and technology. National conferences were held in September on compiling university text-books and, more crucially, on university enrolment. A new periodical, *People's Education*, also started publication in October to serve as a forum for discussing changes in education policy.

One of those changes affects applicants for university places. They are now to take the initiative in putting forward their own names for consideration rather than waiting for selection. Although the universities will continue the "open door" policy of enrolling workers, peasants, demobilised soldiers, and ex-students settled in the countryside, a policy of "walking on two legs" is to be followed, so that direct entry from middle schools is now allowed, and is in fact scheduled to account for about 30% of available places.

In the universities, administrative responsibilities will be delegated by Party branch committees to principals and faculty and department heads, posts which had previously been abolished, but now are being re-introduced ('revolutionary committees'

will be phased out). Entrance examinations which "are necessary in evaluating the political and general educational level of entrants", will also return, being held annually in July or August.

Political education, however, will not be played down; most university students, for example, are likely to spend one month every year living and working with workers or peasants. China continues to encourage expansion of the 'July 21' workers colleges run by factories and mines, and agricultural institutes modelled on the 'Chao Yang' college. They accept peasant-students selected by communes who return there immediately after graduation.

### Enrolling research students

It is repeatedly stressed that "to achieve big progress in science and technology it is imperative to arouse the masses boldly"; but, for science at least, more importance appears now to be attached to "the backbone role of professional scientists". The teaching curricula of many universities are being revised, and much effort is being spent on bringing textbooks up to date.

Universities are apparently intended to serve as centres of both higher education and research. Together with institutes under the Academia Sinica, they are preparing to enroll research students for the first time in ten years. Applications will be received this year until the end of December, and post-graduate training is planned to last three years. 1977 graduates will be allowed to take a proportion of the allocated places.

Young people in China are increasingly urged to devote themselves to science. In the rationalist tradition of Marxism, socialist man is substantially equated with scientific man; an additional persuasion now is the call of the four "modernisations", namely agriculture, industry, defence and, most importantly, science and technology.

In November a science week in Shanghai was attended by over two

*T. B. Tang is at St John's College, Cambridge.*



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million school students. And many leading scientists participated in a meeting of the Chinese Scientific and Technical Association in Peking in August, which was attended by over seven thousand middle school students and teachers of mathematics, physics, and chemistry. Delegates were exhorted to "acquire knowledge to scale new heights in science and our socialist motherland". In a typically Chinese manner, the biologist and popular science writer Kao Shih-Chi composed a poem for the students entitled "Make Science and Technology Serve Our Motherland".

The latest drives in China's educational front are no doubt directed towards economic ends; but they also have political goals. The persistent incentive is not materialistic—though such an aspect exists—but ideological, relying on popular enthusiasm.

This brings us to the position of scientists. The attitude towards professionals and scholars has varied widely in recent times. Up to 1956, intellectuals were allowed to retain the status and privileges traditionally reserved for scholars in China as long as they modified their views to fit the current political mood. From 1960 up to the Cultural Revolution certain Party leaders conceded that academics and specialists were useful whatever their personal ambitions or political consciousness.

After the Cultural Revolution and the advent of the 'gang of four' the policy changed. Intellectuals were considered to be entirely dispensable unless they were "red", which meant usually that they had not been "contaminated" with "bourgeois" ideas abroad, that they had not shown "individualism" by producing scholarly works, and that they were not in prominent positions and earning high wages.

#### Working hard for socialism

Today, according to the *Political Report*, "the overwhelming majority" of intellectuals "are willing to work

hard for socialism and are indeed doing so". Political apathy will not be allowed, and help and encouragement will continue to be given to intellectuals "to remould their world outlook"; at the same time, however, they will be guaranteed the conditions to put their abilities to good use.

The "Circular on the holding of an All-Nation Conference on Science", issued in September, announced the restoration of titles for technical personnel as a means of enhancing their sense of responsibility. The Academia Sinica, for example, now has research assistants, and assistant, associate, and full research fellows. An increase in wages took place at the beginning of October, with scientists—among others—given priority for individual recommendation.

The *Political Report* states that one of the aims of the forthcoming All-Nation Conference is to "commend the pace-setters, especially those scientists and technicians as well as workers, peasants and soldiers who have inventions and innovations to their credit". The practice of awarding orders and decorations to scientists, first introduced in 1956, may also be restored.

The *Political Report* also suggests that the Chinese people should unite with "our countrymen overseas"; it appears that a State Department for Overseas Chinese Affairs may soon be formally set up. The main contribution of ex-patriate Chinese, of which there are about 20 million, will be to act as bridges of friendship; but in particular cases they can do more.

This year, for example, has already seen the visit of C. S. Wu and the three American-Chinese Nobel prizewinners in physics. For Yang Chen-ning it was his seventh trip, and in the last few visits he has collaborated on important research projects. Another collaborative research project by Chinese scientists at home and overseas is the research on the heredity role of cytoplasmic mRNA, in which M. C. Liu and P. Y. Pang from Temple

University took part.

#### International collaboration

Perhaps significantly, the *People's Daily* published on 3 October a poem entitled "A returned scientist". But whether any overseas intellectual wishing to return to China, either short-term, long-term, or permanently, is able to do so remains essentially a political decision.

The international transfer of science and technology takes many forms. The most obvious ones are importing instruments, machinery, and complete plants, and acquiring information through the circulation of journals and reports, and through private communication. But this transfer also takes the form of international movements by scientists and engineers.

Many in the West will have, through direct or indirect contact, felt the expanding number of exchange visits with China. In addition, we have been given to understand that, at least for Chungshan University (situated in Canton and one of the 28 "key universities"), visiting professors may soon be welcomed, particularly from the Second World. And here the consideration will be mainly academic.

Chungshan University has announced that it is arranging joint research projects with the Polytechnic of Hong Kong. This collaboration may be viewed primarily as a social relations exercise, but it is an important step.

Another development, reflecting the emergent liveliness in Chinese scientific circles, is the recent appearance in *Acta Astr. Sinica* of a paper contending that the universe is closed. Apparently contradicting the Marxist dictum that the universe is not only boundless but also infinite, the paper might well have been refused publication, a few years ago.

Recently, there have been many changes in China's science and education policy. Most—if not all—are not reversals of previous ideas, but a renewed emphasis on policies formulated in the era of Chairman Mao. The changes are necessary not only to repudiate the manoeuvres of a group now fallen from power, but also to prepare China for the conditions she is likely to face in the near future.

In developing her science and technology, China as a Third World country has had 28 years of useful experience which constitutes a basis on which she can fruitfully draw for future programmes. She is endeavouring to develop a modern technological society modelled neither on Western countries nor on Russia and is taking a path never previously followed. In China as in the West, however, there are many problems ahead, not the least being the social impact of progress in science and technology. □



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# Looking to the stars

**Professor Graham Smith, the British astronomer, visited China this autumn and returned feeling China needs a large injection of Western expertise**

ANY scientist must be encouraged to hear that his government is in favour of a rapid expansion of his subject, and that adequate resources will become available. It must, however, be a daunting prospect when excellence is required within a few years, especially when a lack of contact with contemporary science, and a long history of neglect and social turmoil, provide a starting point far behind the world's front runners.

The training of astronomers in China has suffered in recent years alongside all areas of university education. Nevertheless astronomy, and especially radio astronomy, is a well favoured subject in contemporary Chinese science. There is both a determination to achieve excellence in at least a handful of research fields, and an unprecedented encouragement of contacts with the West.

On my visit to China in October as representative of the Royal Greenwich Observatory, I was accompanied by Professor Wynne, who was particularly interested in optical design. We therefore not only visited the major observatories but also the major optical institutes and factories, in most of which Wynne's work on computer optimisation of lens design was being adopted.

The main observatories in China are at Shanghai, Nanking, Peking and Kunming. Shanghai Observatory is mainly concerned with measuring time through the use of transit telescopes and astrolabes. It has an astrolabe of novel and advanced design, recently described in *Acta Sinica Astronomica* (an English translation of this journal is now available). Although Shanghai is a centre for the time service in China, it has no caesium clocks, and China takes no part in international efforts to maintain the Atomic Time Scale.

Nanking has the famous Purple Mountain Observatory, where some of the most spectacular of the fifteenth-century bronze instruments are preserved, and some astrophysical work and satellite tracking observations are now carried out. Peking Observatory has several departments, notably the optical observatory at Hsin Lun, 960 m above sea level and 110 km NW of Peking, where there are several working telescopes, including a 60 cm Cassegrain recently built at the Astronomical Optical Factory of Nanking. The same factory is now undertaking the construction of a 216 cm telescope

for Hsin Lun.

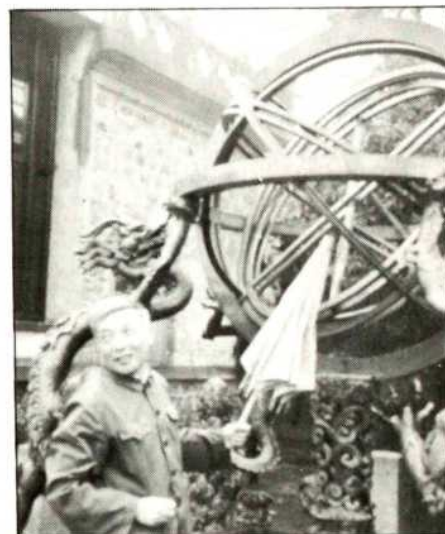
The radio observatory of Peking is at Mi Yun, about the same distance from Peking. The site is well chosen on a flat plain screened by hills from interference, and has a 1 km array of 9 m reflectors. And at Kunming, in the south-west of China in Yunnan province, there are solar and astrometric telescopes, and an active observatory which recently started a large expansion.

Peking University has no research school in astronomy, even though radio astronomers are taught there. Their training has consisted of a third undergraduate year in radio astronomy, in which practical experience with a very elementary solar radio telescope played an important, but hardly advanced, rôle. The modern techniques of aperture synthesis and very long baseline interferometry (VLBI) were often mentioned, but there is naturally no direct experience of such important ingredients of modern radio astronomy, and no plans have yet been formulated of how to start.

The radio observatory at Mi Yun is the obvious nucleus for any expansion in the subject. The 22 paraboloids of the 1 km array have recently been rebuilt, increasing their diameters from 6 m to 9 m, and are used as a phased array at frequencies of 450 MHz and 176 MHz, observing the sun. The angular resolution is to be increased by the addition of further paraboloids, spaced at further intervals of 1 km, and used in a switched system.

The next development planned would be to rebuild the whole system for use in aperture synthesis, although this would be a very large task, involving amplifiers and separate cables for each paraboloid, a new complex receiver system considerably more sophisticated than the present simple switching receiver, and an on-line computer.

My own view is that rapid progress of the necessary order could only be achieved by a determined infusion of ideas and techniques from the world's large radio observatories. The problem of language is serious, since few visitors could even hope to learn Chinese, and surprisingly few of the Chinese astronomers speak English well. The main problem, however, is the lack of students and young research workers with any familiarity with modern work. It is essential for some of the best of young Chinese astronomers to spend considerable periods abroad; possibly two years in a major observatory would



*Fifteenth century astrolabe at Purple Mountain Observatory*

be needed.

Although radio astronomy is at present the favoured subject, progress might be better made by concentrating on optical, or possibly on millimetre wave astronomy. The site of the Peking optical observatory is not particularly good, although it may be the best within reasonable distance of Peking. But there must be some very good sites in China, especially at the high altitude which millimetre wave astronomy demands.

Kunming itself is at 2,000 m, and the skies are clear, while not far from the city are mountains over 2,500 m which might provide good sites. There is undoubtedly a wealth of good astronomy to be done with moderate sized optical telescopes; the worrying aspect of radio astronomy is that middle-sized radio telescopes may be no use in comparison with the giant arrays now being used, or constructed, in a few major observatories.

In the observatories and institutes which we visited there are plenty of people, and there are new buildings which at Kunming reach a lavish scale. There must be room for a staff of at least 500 when the buildings are complete even though the observational work in progress at present would occupy a staff of only about 50 at an established observatory. Optical design is becoming well understood in China, and the techniques of optical working now extend to the figuring of mirrors up to 2.5 m in diameter.

Instrumental design has been well demonstrated in the new astrolabe constructed in Nanking and used in Shanghai and Kunming. Spectrograph design needs study, but gratings are now available which are ruled at Chang-Chun, in northern China. Low expansion glass is now made in Shanghai, although only in pieces up

to 1 m in diameter. (The 216 cm mirror is a piece of pyrex made in Russia, and presumably imported almost 20 years ago.)

There is much enthusiasm and a good-will towards astronomers which is refreshing and helps to make a visit to China an extremely pleasant occasion. The Chinese want to make real

advances, and recognise that to do so they must participate in the international exchange of ideas which is an outstanding characteristic of astronomy.

It would be very helpful to China's scientists—and surely very good for international good will—if astronomers were sent abroad for extended periods

of study. The regeneration of Chinese science without such contacts will otherwise be a slow and uncertain process. Whether it occurs in radio or in other branches of astronomy will only emerge later, when there are sufficient trained young astronomers able to make their own educated choice. □

## Divide between Peking and Taipei rules out scientific cooperation

*The issue of the two Chinas is gradually concerning more scientists as the Peking government seeks membership in the international unions at the expense of Taiwan. Richard Harris, of The Times, who lived in China before and after the Communist government came to power, describes the historical background and explains why a two-China policy is unacceptable to either government.*

IN 1895 China was ignominiously defeated in a brief war with Japan over Chinese suzerainty in Korea, and as a result Taiwan had to be ceded. China felt this defeat by Japan much more shameful than either of the earlier Anglo-Chinese wars in the nineteenth century. Moreover, emotions were stronger in 1895 since by then a slow response to western intrusion had evoked a nationalism never before felt in China.

The two decades following that defeat (which included the actual fall of the Ching dynasty in 1911) brought home to the Chinese the need for their twentieth-century revolution, and defined its objectives. These involved the regeneration of China—the Chinese term used for revolution has this meaning—so that it would again be united, independent and strong.

United meant the return to the sovereignty of one government over all territories from which such sovereignty had been lost by China from 1840 onwards. Independent meant the abolition of any privileges otherwise acquired by foreigners—such as the treaty ports and the customs control exercised in accordance with “unequal” treaties. Strong meant a China militarily and industrially the equal (if not the superior) of any country in the advanced western world.

Ever since 1895 the loss of Taiwan has remained indelibly in the Chinese mind as a shame to be expunged. Along with other causes for shame, the emotions of Chinese nationalism survived unappeased in this century until

the first government strong enough to pursue the national objectives emerged in 1949. As the communist armies moved southwards in that year, all patriotic Chinese welcomed the end of foreign privilege, and foresaw that Chiang Kai-shek's retreat to Taiwan could only be temporary since all his support had gone and his troops were demoralised.

Suddenly, however, in quite unforeseen circumstances, hopes of a united and independent China were dashed with the outbreak of warfare in Korea (not of China's making), and President Truman's decision to place American power between Taiwan and the intended assault on it from the mainland.

Unlike the situation with East and West Germany, the incoming revolutionary government of 1949 therefore found itself facing exactly the same circumstances as earlier new dynasties when pockets of support for the defeated dynasty were still resisting. Such redoubts of resistance had always maintained their own legitimacy; by so doing they challenged the legitimacy of the incoming rulers in Peking.

Moreover those who thought they had seen the end of foreign privilege and foreign interference in the affairs of China now saw just such foreign interference reasserted in favour of Chiang Kai-shek. An American security treaty soon assured the island against attack, while American hostility to the People's Republic for 20 years impeded diplomatic recognition, and the occupation of the Chinese seat in the UN by the Peking government, which was retained for the government in Taiwan.

As seen from the west, much has changed since 1950. Taiwan's economy has shared in the growth area dominated by Japan. The circumstances of the Korean War have been forgotten. Why should Taiwan be “handed over” to the communists, it is asked. Furthermore, now that the United States has changed its policy of containment towards China; and has established a

mission in Peking, why should China not agree to American requests that the government in Peking should renounce any intention of occupying Taiwan by force?

No one with any first hand experience of Chinese nationalism, or knowledge of Chinese history, could expect the Chinese to meet such demands. Consider them in reverse: would the Americans, solely at China's request, be prepared to renounce action of some kind about territory that was part of the United States?

Another argument offered by westerners is to use the German analogy, and to ask why Peking should not be content to allow Taiwan a comfortable independence. Those who suggest this, however, do not realise that the government of the Kuomintang Party in Taiwan shares all the attitudes of the government in Peking. Each government claims to be the government of China; once the government in Taiwan renounced that claim, it would lose its *raison d'être* even if its reconquest of the mainland is an absurd ritual declamation.

Whatever the arguments, there is no possibility that action by the west, by the UN, or by any other outsider can determine the future of this question. And of those who cling to the hope that the “Taiwanese”—who certainly do not like the dominance of Kuomintang mainlanders, and who find that dominance has not been much mitigated by the greater association of Taiwanese in government in recent years—will assert their wish for independence, one can only ask whether the record of Chinese history gives them any support at all.

Foreign intruders have ruled parts of China at many times, and all of it in the case of the Mongols and Manchus (Ching). But at no point has the concept of unitary government for all persons who are Chinese ever been questioned—not even by the warloads of the twenties. Supporters of a “two-China theory” have been, are and always will be anathema to the government in Peking.

This should explain why neither government will entertain any contact with the other, and why the battle between them in all international organisations, from the UN downwards, will allow no co-existence. □



## Israeli scientists woo Egypt

AMONG the documents that Egyptian President Anwar Sadat took back with him to Cairo after his recent extraordinary visit to Israel was a list of 36 international scientific conferences due to take place in Israel this year. Appended to that list was a request that Sadat facilitate the participation of Egyptian scientists in each of these conferences.

A much more far-reaching proposal for scientific cooperation was made immediately after Sadat's departure by Technion Professor Josef Rom, an aeronautical engineer now serving as a Likud member of the Knesset. Rom said that the current Israeli-Egyptian political dialogue should be accompanied by concrete measures to foster joint programmes in as many spheres as possible, among them science and technology. "For example," Professor Rom declared, "we must begin to plan and if possible to carry out bi-national projects such as the construction of atomic power stations and desalination plants in Sinai".

Such a scheme, in fact, was proposed many years ago by Dwight D. Eisenhower, and endorsed by other American leaders, all of whom thought it could help bring peace to the Middle East. Only later did it become clear that in the absence of peace, co-operative development projects were simply not feasible.

But before that scheme was finally shelved, American, Israeli and Egyptian scientists had come together at the Oak Ridge National Laboratory, then headed by Dr Alvin Weinberg, to work out details of how it could be carried out. They envisaged the installation of

two reactors with a combined output of 1,000 MW in the El Arish area, south of the Gaza Strip. The power generated was to be used by newly established industries producing chemicals, fertilisers, plastics, and aluminium, as well as for the operation of a plant to desalinate 1,000 million gallons per day of seawater—enough to irrigate some 300,000 acres of land in the deserts of Sinai and the neighbouring Negev. It was assumed that this American-financed programme would bring great material benefits to both Israel and Egypt and also provide employment opportunities for Arab refugees in the Gaza Strip.

In the interval most of these refugees have obtained jobs in Israel and many other things have changed. But Dr Gerald Stanhill, an Israeli scientist who helped work out some of the agricultural aspects of the scheme, hopes that it will be revived in one form or another.

At the same time, Dr Stanhill warns against the dangers of prematurely promoting "intensive, California-style agriculture" in a country like Egypt because this would involve an enormous investment in money and trained manpower as well as flooding Egypt's already overcrowded cities with "fellahs" who would lose their jobs in the countryside and fail to find new ones in the cities.

But quite independent of the Sadat visit, Stanhill and his colleagues at the Volcani Centre of Agricultural Research have been considering how low-technology irrigation techniques similar to those used on Israeli moshavim (smallholders' settlements) could be

introduced into the Egyptian countryside in a way that would substantially improve living standards without destroying the existing fabric of society. And these techniques—together with those from dozens of other countries—are being brought to the attention of the world's farmers and researchers through the efforts of the International Irrigation Information Centre (IIIC) located at Volcani.

Even so, personal contact is bound to be a more effective means of communication. This can be seen in the Gaza Strip, controlled by Israel since 1967. There Egyptian-trained agricultural instructors were taught Israeli techniques in irrigation and other spheres, and then brought them to local farmers. As a result, average annual production in agriculture has grown by 25.4% (as compared to 6–7% in Israel), while income per farmer has risen from \$130 to \$732.

There is enormous enthusiasm among Israeli researchers—be they working in agriculture, medicine, electronics or geophysics—to establish close contacts with their counterparts in Egypt, by far the largest and most developed scientific centre in the Arab world.

Weizmann Institute President Michael Sela was undoubtedly speaking for many others when he said this week that the Institute "soon hoped to have visiting scientists not only from Berkeley, London, Paris, and Tokyo, but also from Cairo and Alexandria". "Moreover," Professor Sela added, "if our researchers can spend their sabbaticals on the banks of the Thames or the Seine, there is no reason why they shouldn't be spending them on the banks of the Nile".

**Nechemia Myers**

## Euratom burns its public relations' fingers

RELATIONS between the European Commission and the environmentalist lobby got off to a bad start on the first day of last week's public hearings into nuclear energy with the publication of a report purporting to show that the European Atomic Energy Community (EURATOM) was planning a propaganda campaign to sway public opinion in favour of nuclear energy.

The "evidence" for this claim was a report "Design of a Task Force to Build Public Awareness and Support for Nuclear Power", prepared under a Euratom study contract, which was produced at press conferences held simultaneously in Brussels and Rome by the Italian Radical Party and anti-nuclear lobby.

The report, prepared by Alessandra Ovi of the Nuclear Engineering De-

partment at the Polytechnic Institute of Milan, and written in November 1976, described the details of a communication process which would "successfully tackle" public opposition to nuclear power.

Techniques suggested for achieving this included making use of the "authority and credibility" of senior members of the medical hierarchy to diffuse a pro-nuclear message. The report recommends setting up a task force of experts who would "design adequate messages" to provide a coordinated response to nuclear opponents.

The commission's first response to the Italians' charge of manipulating public opinion was to dismiss the document as a "forgery". Later, however, when copies of the document

were produced bearing the EURATOM Study Contract Number 605-76-03, the commission admitted its authenticity.

But according to the commission, the document bore no relation to official policy. It had apparently been a sub-contract made under a much larger contract (to which the contract number referred) on the general assessment of nuclear risk.

The report had been immediately disowned by EURATOM when its existence had become known in June of this year, with firm instructions that the line of inquiry it indicated should not be pursued further. How it got into the hands of the Italian environmentalists is not known; but its very existence, and the swift reaction which it aroused from the commission, was sufficient to add an initial jarring note to the three-day hearing.

**David Dickson**



## New broom at the DHSS

**E**FFORTS to streamline the administration of medical research have been promised by the new chief scientist at the UK Department of Health and Social Security, Professor Arthur Buller.

The promise is likely to be widely welcomed by Britain's medical science community. There has been growing frustration in recent months at the burdens imposed on research by the implementation of Lord Rothschild's proposals for research council funding.

Professor Buller brings to the job a growing awareness that the Medical Research Council (MRC) and the DHSS must work closely together, and with mutual trust, if the new system is to be made to work. Only this, for example, will avoid the DHSS presenting the research council with requests—such as a “cure for schizophrenia”—that are impossible to meet at the present time.

A close liaison will also, Professor Buller feels, avoid a repetition of the situation that arose two years ago when the Government's economic problems led to hastily imposed cutbacks of department research budgets, and consequently a reduction of £900,000 in the money available to the MRC from the DHSS.

“It is impossible to maintain any type of meaningful relationship between the two bodies if this type of thing, which happens when administration gets quite separated from research, is liable to take place. From the MRC's point of view, last time was a near disaster.”

Professor Buller, whose appointment was announced last week, will take up his new position with responsibility for a £28m research budget from the beginning of January. He is keen to reduce the extra administration that the Rothschild proposals have involved, a desire he shares with the new secretary of the Medical Research Council, Professor James Gowans.

Unlike many scientists, however, he does not criticise the philosophy behind the proposals, under which about 25% of the MRC's £52m research budget is now allocated by the department to the research council on a “customer-contractor” basis.

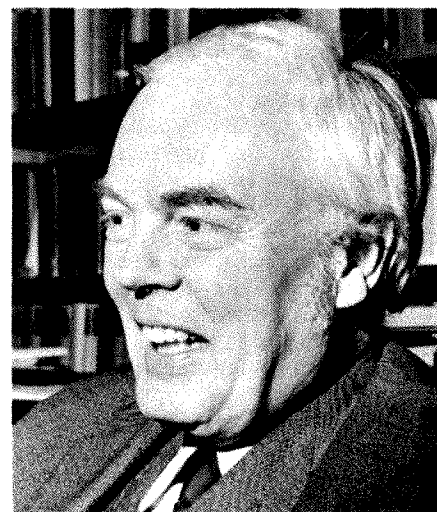
According to Professor Buller, the problem lies in the way that this has been done. “In the past, too much of a meal has been made of being seen to implement Rothschild. An enormous exercise, which has been expensive and time-consuming for both scientists and administrators, has been mounted so that both sides could demonstrate that they were carrying out Rothschild's recommendations”, he says.

By cutting down on this type of activity, while concentrating on what remains necessary to maintain an effective partnership between the MRC and the DHSS, Professor Buller hopes to simplify the administrative system between the two bodies, without any loss of effectiveness. And it is a goal which, he claims, is already shared by the DHSS.

Professor Buller has been seconded to the department from the University of Bristol, at which he is professor of physiology and dean of the faculty of medicine. He shares a general reputation claimed by the university for pragmatism rather than politics in administrative affairs, although membership of the non-academic staff salaries committee has made him no stranger to the latter.

Buller gained further experience in the world of medical politics at the MRC itself, where he is at present chairman of the neurobiology and mental health board and a council member. The board's main concern in recent years can, he says, be succinctly summarised: “We have spent our time trying to live with Rothschild”.

One of Buller's aims likely to receive widespread support among scientists is his desire to return the initiative on research back to the research worker. The most important thing, he stresses, in avoiding the waste of money is to get good scientists doing research, and then



*Arthur Buller, new chief scientist*

to a certain extent to follow their judgements.

The real need is not the development of ways of directing research from outside, but the successful application of its findings. “We need to keep a very sharp eye on ways in which the research being done by scientists in ivory towers can be exploited for the improvement of health care”, he says.

“In the past a number of important discoveries have lain fallow and not been exploited. Penicillin is an example where a failure to pick up the possibilities early enough meant that the industrial lead was given to the US. I am very keen to avoid this type of thing in the future”.

**David Dickson**

## Indian science reorganised

**T**HE Indian government has now issued a list of the 17 laboratories that it intends to detach from their present position with the Council of Scientific and Industrial Research (CSIR) and relocate within user ministries (10 November, page 89). They are: ten research associations (to Industry), three museums (to Education), the Indian Institute of Petroleum (to Petroleum and Chemicals), the Road Research Institute (to Transport and Shipping), the Central Fuel Research Institute (to Energy) and the Building Research Institute (to Welfare and Housing).

In contrast to the earlier fears that the government would dismember CSIR and shatter morale, first reactions seem to be relief that the transfer has only affected laboratories most easily housed in a ministry. □

## Following a Maoist path

Romanian science is being systematically ruined “on the Chinese model”, according to Dr Mihai Dediú, a Romanian mathematician recently exiled with his family to Italy after his cam-

paign for genuine scientific activity.

Although throughout the Comecon block social doctrine states that science must serve the economy, in most states this is no more than a slogan; pure research continues following Brezhnev's dictum that “there is nothing more practical than a good theory”. In Romania however, following President Ceausescu's visit to China, a new trend began by which all institutes of pure research were gradually turned into what Dr Dediú describes as “little more than factories”.

Chemistry was the first to suffer—there is now no faculty of chemistry at all, only chemical engineering. The mathematics institute was destroyed in 1975 and some of the mathematicians were transferred to the Institute of Physics, some to industry. The Institute of Physics, in its turn, became the Institute of Physics and Instrument Production in autumn 1976. According to Dr Dediú, the Academy of Sciences no longer exists as a scientific forum. The whole emphasis is upon politics. Even Madame Eleana Ceausescu, the wife of the leader, has been appointed an academician although she has no scientific training.

Dr Dediu himself was involved in these changes, being employed at the Mathematics Institute at the time of its destruction. He was then transferred to the Physics Institute, but was dismissed before it was re-modelled.

**Vera Rich**

## Change into genes

Britain's House of Commons Select Committee on Science and Technology is to set up a subcommittee on genetic engineering under the chairmanship of Mr Arthur Palmer, Labour MP for Bristol North East. The members of the committee will be announced shortly, and it is expected that the subcommittee will begin public hearings—to which Ministers are likely to be invited to give evidence—early in the new year.

## Conserving energy

THE European Commission in Brussels is considering plans to set up a group within the energy directorate concerned directly with the problems of energy conservation.

The suggestion for such an initiative was made by various environmentalist groups during the public hearings on nuclear energy held in Brussels last week, the first of a series of such hearings on issues related to energy policy.

At the end of the three-day hearings, the chairman Dr Guido Brunner, who is commissioner for energy, announced that the proposal would be seriously considered by the commission, and that it was likely to be put into effect.

The hearing, which concentrated particularly on future energy requirements, saw a number of confrontations between the pro- and the anti-nuclear lobbies, with many well-worn positions being rehearsed by either side.

Putting the case for nuclear power, for example, Dr Rudolf Guck of Badenwerk AG suggested that the declining supply of fossil fuels, leading to a steady climb in prices, would make nuclear energy an increasingly-attractive economic proposition.

According to Dr John Chessire of Sussex University, however, current forecasts of future electricity demand were too high. In addition, whatever the price of uranium, fast breeder reactors would, he claimed, be uneconomic compared with thermal reactors.

Criticism of the European Commission's own energy policy as being "obsolescent" and ignoring "the real needs of society" came from Dr Peter Chapman, director of the Energy Research Group at Britain's Open University.

In a presentation that appeared to make a particular impact on Dr

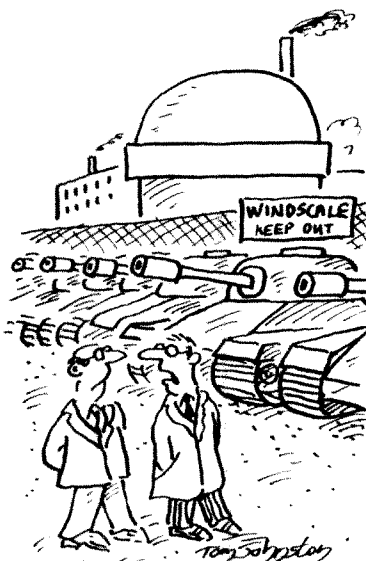
## Prophet of nuclear doom

Mr Tony Benn, UK Secretary of State for Energy, appeared to enjoy last Friday's debate in the House of Commons on nuclear energy.

The debate was brought by Pontypool MP Mr Leo Abse, whose constituents, he said, live within 50 miles of "one of the largest concentrations of nuclear reactors in the world . . . eight reactors in operation . . . and two more planned".

Abse, who had been briefed by Tom Burke of Friends of the Earth, was demanding a government response to the Flowers report, which indicated the dangers of a headlong dash to dependence on nuclear power. Not mincing words, Abse raised the question "is a plutonium based economy compatible with democracy?" and addressed it with some eloquence.

Abse is not exactly pro-nuclear. He



'UNFORTUNATELY, MOST OF THE PLUTONIUM WE PRODUCE GOES TO POWER OUR SECURITY SYSTEM.'

ended "If we, avaricious for the apparently easy wealth that nuclear energy could bring, enter into a Faustian pact with a meretricious atomic destiny, we may have doomed our children or grandchildren to the loss of their liberties, if not their lives".

But that style is very much Mr Abse's. In more sober moments he took a view broadly against plutonium,

but not against nuclear energy as such. His points were cogent—as indeed are many of those raised by the Friends of the Earth. Tellingly, Benn was delighted with Abse's speech, which he thought "in a classic prophetic mould". Abse's, said Benn, was a "philosophical, penetrating, and perceptive speech, and I hope that it will be widely read and studied". Benn was at pains to point out that in matters of such difficulty it was worth listening to everyone and that "the pressures that are brought to bear are not—as is sometimes suggested—only those brought by the environmental lobby against the innocent nuclear power lobby". Benn went on "In my political life I have never known such a well-organised scientific, industrial, and technical lobby as the nuclear power lobby. It is not so much the Friends of the Earth as what Eisenhower might have called the nuclear industrial complex of which I am aware as a Minister."

With that view, one might have expected Benn to be more forthcoming on the question of an inquiry into the proposed building of a commercial prototype fast breeder reactor in the UK, the £2 billion CFBR1. Mr Peter Shore, Secretary of State for the Environment, has promised a fast breeder inquiry separate from the recent Windscale planning inquiry, which investigated the extension of reprocessing facilities at Windscale. Asked if the government had indeed committed itself to an inquiry on the fast breeder Benn said "We have not yet decided to set up the inquiry". And yet he added "We shall not reach a decision (on the CFBR1) until there has been an inquiry".

Mr Tom King asked for confirmation that the government would not take a decision on fast breeders until the inquiry, and suggested that the Minister had not even discussed the question of an inquiry with other Ministers. Is there progress towards an inquiry, he asked? Benn, apparently cornered, answered "I cannot undertake that there will be a debate . . . when we have settled the thermal reactor question I shall be better able to answer".

**Robert Walgate**

Brunner and other members of the commission present, Dr Chapman claimed that the commission's policy was at least four years behind that of the UK, and that the supply and demand forecasts which it had been putting forward were inappropriate, and of a "very low standard".

"There seems to have been no

analysis of the economics of nuclear systems and no awareness of the importance of storage and transport costs", said Dr Chapman.

The next public hearing, which will be held in Brussels on 25 January, will discuss some of the problems raised by the safety aspects of nuclear energy.

**David Dickson**

# correspondence

## What about the peace prize?

SIR,—I am not surprised that, in the normal way, the only Nobel Prizes to which you pay attention (27 October, page 744) are the prizes for physics, chemistry and physiology and medicine. This year, however, scientists have cause to take particular pleasure in the award of the Peace Prize. The 1977 Nobel Prize for peace was awarded to Amnesty International, who—as your own columns have not infrequently reported—have often been involved with helping scientists among the many prisoners of conscience for whom they work.

J. A. EADES

University of Bristol, UK

## Limited tenure

SIR,—Both the present Director of the Imperial Cancer Research Fund, Dr Michael Stoker, and his successor, Professor Walter Bodmer, are on record as believers in “the virtues of limited tenure as a means of maintaining high quality in research”. These are fine, high-sounding epithets, but what they actually mean for scientists carrying out research during short contracts is something totally different.

The majority of indigenous talent in cancer research in England is recruited at the doctoral or immediately post-doctoral levels; very often the “virtues of limited tenure” brutally mean—get out by the age of thirty, or certainly not much later. Nobody engaged in activities other than scientific research in the same institute, from the administrators to the catering staff, receive such cavalier treatment; indeed, it would be against the law. Often the PhD student or post-doctorate will feel it necessary to justify himself to his superiors by working long hours, to the detriment of his social life, if not his health. Certainly this will maintain high output, if not also “high quality in research”. In addition, the financial return compares badly with many other professions, quite apart from such incidental mishaps as the refusal of building societies to grant mortgages because an income cannot be guaranteed for the appropriate period of time. And of course the searing intellectual corrosion, the shame, the agonising losses of self-confidence and self-respect that characterise the phase of unemployment and readjustment—inevitable in

many cases—these are the price each individual has to pay for having done a highly skilled job cheaply and to the best of his ability.

It is perhaps unfair to single out the ICRF for blame in a situation in which the market for research scientists is presently glutted by talent of an exceptionally high order in virtually all scientific disciplines; in which the governing principles of employment in grant-aided research for those not medically qualified are primarily cheapness and expendability as opposed to merit or achievement. It is easy to spread an ambivalent gloss over the realities of the situation by glamorising the advantages of short-term appointments as a means of achieving academic excellence, though even this is a highly questionable thesis. Conversely, it is perniciously wrong that gifted young people, often guided into cancer research for reasons that have nothing in common with self-advancement, should be called upon to pay such a bitter and a heavy price for their altruism.

ROBERT JONES

Tübingen, West Germany

## Funding of basic research in chemical thermodynamics

SIR,—The membership of the 1977 Calorimetry Conference is deeply concerned with the continuing decrease in funding of research in chemical thermodynamics, especially thermochemistry and thermophysics, and the resultant decrease in data productivity in this field in the past several years. Examination of publications and of Calorimetry Conference programmes shows the greatest decrease to be in the areas of heat capacity measurements and of heat effects in chemical reactions (combustion calorimetry, solution calorimetry, etc.). These are the primary sources of new thermodynamic data for science and technology.

In North American universities, experimental expertise is in danger of being lost in important areas, particularly combustion calorimetry. Programmes in heat capacity calorimetry and in reaction-solution calorimetry are also suffering. Attention is called to the following points:

The production of fundamental thermophysical and thermochemical data for new materials, and for materials not previously

studied, is not now sufficient for the evaluation of new processes and applications. Many key areas are no longer being studied. These fundamental data are required for effective research and development in virtually all aspects of energy and technological materials programmes.

The precise determination of the appropriate properties of carefully selected ‘key compounds’ is important because the data provide anchor points for making critically evaluated estimates and correlations of the properties of whole classes of compounds. This allows the data base to be expanded greatly on the basis of a relatively small number of carefully chosen measurements.

Acquisition of new data of technological significance on a realistic time scale requires continuous funding to keep available the highly sophisticated equipment and manpower in laboratories necessary for energy-related measurements, in other words, to maintain capability. The initial investment that has already been made could be lost if sufficient funding is not continued.

Continued acquisition of fundamental calorimetric data has been demonstrated as an important endeavour in the solution of practical problems in many areas of science and technology. It is an economical endeavour, leading to increased efficiency in the choice of materials, the design of production facilities, energy conservation, and the selection of appropriate large-scale technology.

Fundamental thermodynamic data are also necessary for the critical testing and evaluation of theoretical advances in chemistry.

The conference urges that governmental, private, industrial, and university laboratories, in the light of the above comments, consider their anticipated needs for chemical thermodynamic information and identify the level of support required (manpower and facilities) to supply the information at a rate commensurate with the need.

Particularly where substantial funding reduction has occurred—perhaps originally as a temporary measure—consideration should be given to restoring support on a continuing basis.

Assurance of multi-year support rather than a strict year-by-year support is recommended because of the resulting economies and because the production of high quality data requires a continuity of trained and experienced experimentalists.

Yours faithfully,

CHARLES E. HOLLEY

Calorimetry Conference Committee,  
University of California, USA

# news and views

## Ribosomal gene organisation in *Tetrahymena*

from Carol Klukas

THE finding of a single copy gene for rRNA in the micronucleus of the protozoan *Tetrahymena* by Yao and Gall (*Cell* **12**, 121; 1977) is the latest result of their investigation into the structure and organisation of the rRNA genes in this organism. It leads the authors to suggest that *Tetrahymena* may represent an evolutionary linkage between eukaryotes and their prokaryote-like ancestor.

*Tetrahymena*, a ciliated protozoan, contains a germinal diploid micronucleus which maintains the genetic continuity of the organism, as well as a polyploid macronucleus, which is formed after conjugation from the zygotic nucleus. The macronucleus functions as a somatic nucleus directing most of the cell's transcriptional activity during vegetative growth, but is destroyed at the end of each sexual generation. By DNA-RNA hybridisation using the 25S and 17S rRNAs of *Tetrahymena*, Yao *et al.* had previously shown that although 0.3% of the DNA in the macronucleus (which contains 23 times as much total DNA as the micronucleus) codes for rRNA (about 200 copies per haploid genome) only about 0.03% of the micronuclear DNA is rDNA (*Proc. natn. Acad. Sci. U.S.A.* **71**, 3082; 1974). Moreover, the vast majority of the rDNA in *Tetrahymena* exists as extrachromosomal pieces, about  $13 \times 10^6$  daltons, each containing two copies of the rRNA genes arranged in a palindrome (Karrer & Gall, *J. molec. Biol.* **104**, 421; 1976; Engberg *et al.* *J. molec. Biol.* **104**, 455; 1976).

Considering the micronuclear origin of the macronucleus and the 10-fold difference in the rDNA content of the two nuclei, it is reasonable to suggest that the extrachromosomal rDNA of the macronucleus arises by amplification from one or a few rDNA copies integrated into the germinal chromosomes of the micronucleus. Working

from this hypothesis, Yao and Gall have tried through their experiments to establish the molecular basis of this amplification, asking whether or not the rRNA genes are in fact integrated into the micronuclear genome and if they are, do they have the same palindromic structure as in the extrachromosomal rDNA in the macronucleus, and furthermore are there any rDNA copies integrated into the macronuclear chromosomal DNA?

To answer the first question Yao and Gall digested macronuclear and micronuclear DNA individually with restriction endonucleases. *EcoRI* cleaves *Tetrahymena* rDNA at one site yielding two classes of fragment ( $10 \times 10^6$  and  $1.6 \times 10^6$  dalton) from the extrachromosomal rDNA palindrome. Yao and Gall digested micronuclear DNA with *EcoRI* and identified the fragments containing rDNA sequences by radioactive cRNA probes. They found the  $10 \times 10^6$  and  $1.6 \times 10^6$  dalton fragments (probably as a result of unavoidable contamination with macronuclear rDNA) but also a third fragment of  $5.3 \times 10^6$  daltons. They interpret this to be derived from one end of an integrated rDNA gene together with its flanking chromosomal sequences. This fragment appeared as a band of very low intensity suggesting that a very few copies at most of this integrated structure exist in the genome, most probably only one.

By analogous restriction enzyme mapping experiments with *BamHI* and *HaeIII* Yao and Gall show that in fact the *EcoRI* digestion of the integrated gene also yields a second fragment of  $10 \times 10^6$  daltons. This was not recognised in the first instance because it comigrated in the agarose gel with the largest of the extrachromosomal rDNA. *EcoRI* digestion products which contaminated the preparation. These two  $10 \times 10^6$  dalton fragments are not identical, however. In extrachromosomal rDNA the fragment is palindromic so that digestion with additional restriction endonucleases is always symmetri-

cal. In microsomal rDNA subsequent digestion is not symmetrical, indicating that the  $10 \times 10^6$  dalton piece includes only  $5 \times 10^6$  daltons of rDNA, the other half being the piece of chromosomal DNA immediately adjacent to the integrated gene. Yao and Gall conclude that the rRNA gene integrated into the micronuclear genome of *Tetrahymena* exists as a single copy and not in the palindromic structure observed in the macronucleus nor in a tandem array.

To determine whether there are any integrated rRNA genes in the macronucleus the same digestion experiments were carried out on macronuclear chromosomal DNA as free as possible from contaminating extrachromosomal rDNA. No digestion products containing rDNA sequences (except for the bands attributable to the contaminating extrachromosomal rDNA) could be seen. Thus it is probable that no rRNA gene is integrated into the chromosomal DNA of the macronucleus.

These findings lead directly to the conclusion that the extrachromosomal rDNA palindromes of the macronucleus must be derived from the single integrated rRNA gene in the micronucleus. Yao and Gall propose a very plausible model for this synthesis and amplification assuming that there exists at the end of the integrated gene a palindromic sequence just long enough to allow branch migration to occur. This would provide a primer for the replication of the integrated rDNA sequence and the newly synthesised sequence could eventually be released from the chromosome as one folded DNA chain which could then replicate semiconservatively to generate the palindromic molecule observed in the macronucleus.

The finding of only one or a few chromosomally integrated rDNA sequences in the micronucleus of *Tetrahymena* is reminiscent of the low multiplicity of rDNA in prokaryotes and in the organelles such as mitochondria of higher eukaryotes; how-



ever, there is no other eukaryotic system in which rDNA has been found integrated in the nuclear chromosomes as a unique sequence.

But perhaps in the case of *Tetrahymena* this situation seems quite reasonable. The micro and macronuclei can be considered analogous to the germinal and somatic cells of higher eukaryotes except that, with the two nuclei occupying the same cell, the micronucleus does not have to direct the synthesis of ribosomes to support

itself. The macronucleus can supervise the household functions such as transcription and translation. Perhaps in higher eukaryotes where the somatic and germinal functions occur in separate cells it is necessary to have multiple rDNA genes integrated into the germinal chromosomes in order to achieve the rRNA synthesis needed to maintain the germinal cell. Hence, '*Tetrahymena* may represent an evolutionary linkage between eukaryotes and their procaryote-like ancestor.' □

## Electron diffraction up to date

from A. Howie

A conference on Electron Diffraction was held at Imperial College, London on 19–21 September, 1977.

FEW fields can surpass electron diffraction in illustrating the disparity in the pace of scientific revelation and the more measured tread of technological change. The fiftieth anniversary of the subject, marked by the conference, proved to be a timely occasion to discuss the recent work which has at last realised the promise held out by the initial experiments. Although the two main active branches of the subject stem directly from the original low energy reflection experiments of Davisson and Germer and the high energy transmission experiments of Thomson and Reid, progress in both cases has been dependent on major instrumental technology such as the development of electron microscopes and ultrahigh vacuum equipment. As emphasised by T. Mulvey (University of Aston) in an entertaining review of the early work, formidable problems of specimen preparation had also to be overcome.

The high energy work is now concentrated in electron microscopy which has become a key discipline both in materials science and in biology and depends (as in the Abbé theory of the optical microscope) on the transmission diffraction imaging process. The diffraction contrast method, most widely used in materials applications, does not resolve the atoms directly but can provide images of crystal defects at a resolution of 1.5 nm (with weak beam methods) and an accuracy of  $2 \times 10^{-3}$  nm in the atomic displacements. It is particularly suitable for structures like metals with small unit cells and was described by Sir Peter Hirsch (University of Oxford) who concentrated on the detailed way in which

dynamical theory can explain the observations both qualitatively and quantitatively. Improvements in electron microscope performance have stimulated considerable activity in direct lattice imaging, discussed by J. M. Cowley (University of Arizona) with reference to the study of complex oxides and related materials with large unit cells. The information about the projected structure obtained in this way is of great significance in numerous chemical and mineralogical applications as well as in biology.

Despite the activity in real space imaging, all interest in the transmission diffraction pattern itself has not been lost, particularly for the study of diffuse scattering in highly disordered materials. In addition, the use of convergent beam diffraction patterns taken from small areas of crystalline specimens using focused illumination and pursued for many years by P. Goodman and colleagues at CSIRO Melbourne, has become a much more routine technique with better vacuum and other instrumental improvements. A major contribution to the meeting by J. W. Steeds and his team (University of Bristol) showed how local information about structure, composition and lattice parameter can be conveniently obtained from convergent beam patterns taken along crystallographic zone axes. It can be predicted that this technique will be increasingly popular, particularly in the context of scanning transmission microscopes where extremely small areas can be probed.

The increasing use of zone axis orientations, previously avoided because of the large number of diffracted beams excited, was perhaps the best evidence presented of the mounting confidence in the use of high energy diffraction theory in such situations. This has followed increases in computing power and efficiency enabling features of electron microscope images

to be interpreted more and more quantitatively with various plane wave formulations of dynamical theory but has also been helped along recently by several useful tricks borrowed from molecular and solid state valence electron theory. Indeed for the band theorist, high energy diffraction theory has emerged as an exciting new two-dimensional game with a ball of variable mass whose behaviour becomes classical in the limit of tight binding.

Electrons rarely scatter only once, particularly in the low energy electron diffraction (LEED) range. The development of a successful dynamical diffraction theory has therefore been even more crucial in the recent use of LEED for detailed studies of surface structure. Probably the key here was the recognition about 1969 of the importance of inelastic scattering described by an optical potential which is now fairly well understood over the whole energy range, even if some basic questions such as the treatment of thermal diffuse scattering or the use of the Doby Waller factor in LEED may require more study. S. Y. Tong (University of Wisconsin) gave an excellent survey of the recent results on surface structures. About 80 structures have now been determined including a considerable number of gas-covered surfaces so that the stage of looking for trends and general principles has now been reached. The fit between theory and experiment, though not quite perfect, is most impressive and generates considerable confidence that the structures deduced will be confirmed when tested by other methods such as X-ray diffraction, in the case of sufficiently perfect underlying crystals, or angle-resolved Auger emission and similar techniques which lean on LEED for their interpretation. More speculative topics on which some progress was reported were the use of spin-polarised LEED and of surface resonance effects when a diffracted beam is parallel to the surface.

Surface structure can also be studied by reflection high energy electron diffraction (RHEED) at glancing angles. J. B. Pendry (Daresbury Laboratory) described efforts to extend LEED theory to this case using the 'chain method' where scattering from atomic rows is treated as a cylindrical problem. The ultimate advantage of electron diffraction over X-ray diffraction, the ability to form real space images, can be pressed home much more easily at these energies, particularly using scanning electron microscopy. It looks probable, therefore, that yet another development in instrumental technology will eventually allow electron diffraction to extend its steady progress to the detailed study of real surfaces. □

A. Howie is a Lecturer in Physics at the University of Cambridge.

## Energetics and antibiotic uptake

from J. R. Saunders and  
Venetia A. Saunders

ALTHOUGH the modes of action of most antibiotics are well established, much less is known of the processes by which such drugs are taken up by bacteria. In a recent paper (*Antimicrobial Agents Chemother.* **12**, 163; 1977) Bryan and Van Den Elzen propose a mechanism to explain the transport of one particular group of antibiotics, the aminoglycosides.

The accumulation of streptomycin (or gentamicin) by bacteria can be divided into three phases. The initial phase is energy-independent and apparently involves ionic interaction of drug molecules with the bacterial surface. This is followed by an energy-dependent phase (EDP-I), which occurs before inhibition of protein synthesis by the antibiotic. In cells that are sensitive to streptomycin, but not those that are ribosomally resistant, this is followed by a third phase (EDP-II) which is also energy-requiring. This phase marks the onset of inhibition of protein synthesis and loss of cell viability and is characterised by a higher rate of streptomycin accumulation than EDP-I.

Bryan and Van Den Elzen have found that mutant strains of *Escherichia coli* deficient in haem production, ubiquinone production or active transport mechanisms accumulate less streptomycin or gentamicin than corresponding parental strains. In addition, such mutants show increased resistance to those and most other aminoglycosides. But they exhibit an increased susceptibility to the aminocyclitol antibiotic spectinomycin. This corresponds quite well with the fact that this drug is more effective than the aminoglycosides against anaerobic bacteria. Increased uptake of radioactive streptomycin was observed in a mutant that is unable to couple oxidative phosphorylation to electron transport (Unc<sup>-</sup>) and is also found defective in membrane-bound ATPase. It is interesting that this strain is hypersensitive not only to aminoglycosides but also to spectinomycin.

If streptomycin is transported into bacteria by means of a specific carrier system then compounds of closely related structure might be expected to compete with it. This is apparently not the case, but accumulation of

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## How many species?

from A. Hallam

A LIVELY controversy has grown up among palaeontologists in recent years about whether marine invertebrate species numbers, or richness, increased systematically through the Phanerozoic or whether an equilibrium situation has existed, with the apparent increase through time being an artefact. The leading advocate of the first view is J. W. Valentine, who has argued for an order of magnitude increase from the early Palaeozoic to the late Cainozoic, while the steady state alternative has been propounded principally by D. M. Raup.

Raup (*Paleobiology* **2**, 289; 1976) estimated the number of species described for each of the geological periods by tabulating new species reported in the *Zoological Record*, and established a strong correlation between apparent species numbers and the present areal distribution of rocks per system. Now Sheehan (*Paleobiology* **3**, 325; 1977) has come up with another interesting finding, having made a study of those palaeontologists expressing interest in fossils of particular systems, using data from the *Directory of Palaeontologists of the World*. It turns out that there is an excellent correlation ( $r = 0.94$ ) between the number of carefully defined 'palaeontologist interest units' and the number of described species per geological period. Sheehan concludes that the total numbers of described species do not seem to reflect meaningful estimates of the original diversity.

In his reply to Sheehan, Raup acknowledges the good correlation established between the number of species described and the number of interested palaeontologists, as well as outcrop area, but maintains that nothing very positive can be inferred

from this. He prefers the view, though he cannot prove it, that geological systems with more available rock have more species and hence more species are described. In other words most palaeontologists are attracted to the most fossiliferous rocks!

Is there any way out of this impasse? Apparently there may be, provided a distinction is drawn between assemblages from different environments, which can be compared from period to period. This has been attempted by Bambach (*Paleobiology* **3**, 152; 1977), who analysed data from hundreds of fossil assemblages from three different types of environment, as inferred from the facies. The high stress, marginal marine environment always has the lowest faunal diversity and the open marine environment the highest, while the variable nearshore environment has intermediate values. Bambach's data seem to indicate that within-habitat variation in species numbers is small for long intervals of time, and that the number of species has increased by a factor of about four since the mid-Palaeozoic, with variable nearshore environments showing a less pronounced increase than the open marine, while the high stress environments show no significant change. Thus Valentine seems to be partly vindicated, but substantial problems of interpretation remain, notably the factors controlling within-habitat species richness. Bambach speculates that changes through time of availability of food resources might be the most significant, but this is clearly an area demanding much further study.

A. Hallam is Professor of Geology in the University of Birmingham.

aminoglycosides in either whole bacteria or spheroplasts is antagonised in the presence of divalent cations. This could imply that these antibiotics are taken up by a carrier for such ions. Unfortunately no system capable of carrying a wide range of divalent cations has been described. A further significant finding is that calcium ions are less effective in inhibiting streptomycin uptake in a ubiquinone-deficient (*ubiD*) mutant than in the wild type. This indicates that ubiquinone has a direct role in the transport of this antibiotic. There is indeed a very good correlation between susceptibility to aminoglycosides and the possession of respiratory quinones. Thus obligate

anaerobes such as *Clostridium perfringens* which have no respiratory quinones are resistant to aminoglycosides. On the other hand, aerobic organisms possessing ubiquinone or menaquinone are usually sensitive to these drugs. Furthermore, aerobically grown cells of *E. coli* are many times more sensitive to these drugs than are anaerobically grown cells. This suggests that uptake of aminoglycosides is dependent on energy derived from aerobic metabolism.

A simple explanation for the data obtained would be that aminoglycoside transport requires carriers that are dependent on trans-membrane proton motive force. However, in the absence

of an identifiable carrier for such antibiotics Bryan and Van Den Elzen propose an alternative hypothesis. They envisage that EDP-I represents transport of aminoglycosides across the cytoplasmic membrane by a process involving "the aerobic membrane energisation complex". The respiratory quinones probably act directly by binding streptomycin and transporting it across the membrane. A further but less likely possibility is that these quinones act indirectly by activating another component of the complex or a transport protein. EDP-II occurs

because membrane-bound ribosomes have a higher affinity than the transport system for streptomycin. Sensitive ribosomes consequently act as a sink for accumulating drug molecules. This would explain why EDP-II is lacking in strains which are ribosomally resistant to streptomycin. The exact mechanism of aminoglycoside uptake requires verification, but the insights provided by this work should help in the design of aminoglycosides and possibly other antibiotics with increased penetrability. □

## Serum albumin structure and function

from Michael Geisow

ALTHOUGH serum albumin is one of the most familiar and abundant proteins, many aspects of its structure and function remain obscure, although knowledge of more exotic proteins has frequently reached the stage of detailed molecular models. In consequence it is pleasing to be able to report recent developments which give a clearer picture of this much studied, but poorly understood protein.

Roles traditionally assigned to albumin include the transport of fatty acids and other metabolites in plasma, although a host of minor functions continue to be suggested and discussed. Albumin is principally synthesised in the liver and recent work has brought its biosynthesis into line with that of other secretory products of cells. Albumin exists intracellularly with a six amino acid  $\text{NH}_2$ -terminal extension. As in the case of proinsulin, this proform is actually made with a further  $\text{NH}_2$ -terminal 'pre' peptide, which is removed at the sites of synthesis.

The impetus for much new work on albumin stems from the complete sequences of bovine (581 amino acids) and human (582 amino acids) albumins. These are the considerable achievements of J. Brown at Texas University

and B. Meloun of the Czechoslovak Academy of Sciences. The primary structures of other animal albumins are well under way. A striking feature of these sequences is the repeating double-loop motif formed by crosslinks from adjacent cysteine residues (Figure 1). These define three domains or homologous regions of chain (marked I to III in Fig. 1). There are strong amino acid sequence similarities between these domains.

It has become usual to assume that homologous regions of polypeptide form closely similar three-dimensional arrangements. This assumption, together with the considerable folding restrictions imposed by 17 disulphide bridges, has led Brown to construct a plausible model for the albumin domain (11th FEBS Meeting abstracts). His conceptually simple structure consists of six  $\alpha$ -helices (three double loops) which form a roughly cylindrical domain. Albumin is made up of an association of three such cylindrical domains.

The obvious repetition of structural features has led to proposed evolutionary pathways for albumins (Brown *Fedn. Proc.* **35**, 2141; 1976; McLachlan & Walker *J. molec. Biol.* **112**, 543; 1977). Both groups agree that the modern protein descended from duplications of a single double loop struc-

ture. Brown suggests from implied structural evidence that myoglobin and albumin share a common ancestor. McLachlan on the other hand believes that this homology results from convergent evolution of albumin and the globins.

Whatever the ancestry, it seems relevant to ask what function the primitive albumin fulfilled, since the much larger modern protein must represent a sophistication of the original idea. Some answers to this question may have been supplied already by the study of the properties of albumin fragments. For some time workers have known that limited proteolytic treatment of native albumin produces large fragments, evidently by cleavage at unstructured parts of the molecule. The recent sequence information has enabled albumin fragments to be accurately located and released these studies from relative obscurity. Many workers, particularly from T. Peter's group at the Mary Imogene Basset Hospital, New York, have found that individual domains or series of double loops result from mild enzyme treatment. Such fragments are well ordered and may largely retain the conformation they assumed in the intact albumin molecule (Reed *et al. Biochemistry* **14**, 4578; 1975). Even more significantly, fragments retain binding sites associated with the native protein, so it is now possible to allocate these to particular regions of the sequence. On this evidence it seems very likely that the primitive albumin, a double loop, provided a useful binding function in some primordial plasma. Perhaps the evolutionary pressure to extend the number of double loops was strong, not merely to bind other ligands, but also to prevent losses from an equally primitive kidney!

Brown's domain model provides an idealised concept of an albumin-binding site. The cylindrical centre of each domain is a space into which project hydrophobic side chains from the helices. The entrance to this space, formed by the tips of the long disulphide loops in Fig. 1 is lined with positive charges. Lysines and arginines seem to cluster at these points. Such a hydrophobic cavity with localised positive charge is tailor-made for the organic anions which occur so frequently as albumin ligands. It is quite certain that the basic side chains in this site model become labelled when reactive ligands are bound to albumin (Gambhir *et al. J. biol. Chem.* **250**, 6711; 1975). Interestingly, albumin domains seem to have developed some functional specialisations. Fatty acids bind most avidly to domain III while bilirubin and other aromatic ligands prefer domains I and II. Such close structural homology, yet diverse bind-

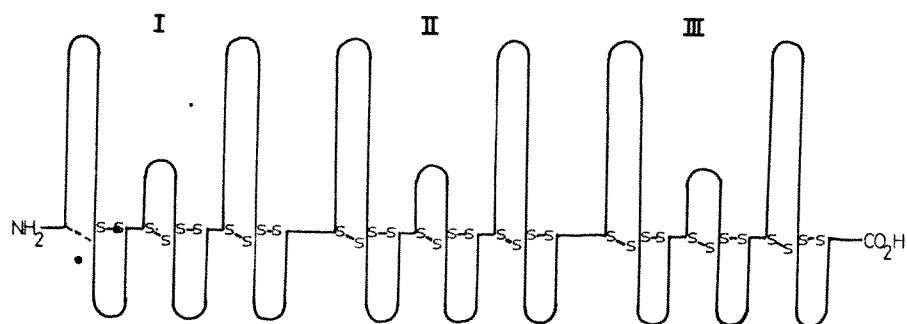


Fig. 1 The double-loop structure of serum albumin.

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ing properties, calls to mind the diversification of the immunoglobulin domains.

Albumin recently joined the list of allosteric proteins, with reports that its binding sites were not independent. The binding of a spin-labelled dinitrobenzene is enhanced by 80% when fatty acids are added to albumin and spin label. The spectrum of the bound label also changes, indicating an alteration of its protein environment (Soltys & Hsia *J. biol. Chem.* **252**, 4043; 1976). The affinity of bilirubin for albumin is also increased upon fatty acid binding (Broderson *11th FEBS Meeting*). Arthur Spector reported at the same meeting that fatty acid, presumably bound to domain III, affected spectral properties of an amino acid solely found in domain II. Some caution is still required, since at moderate concentrations most albumin ligands bind at more than one site and it may be difficult to distinguish between cooperative effects and a more simple direct competition. However, Broderson, Torgny and Sjöholm (*J. biol. Chem.* **252**, 5067; 1977) have

shown that albumin does independently bind the antianxiety drug diazepam and bilirubin. These two do not share the same site and the calculated coupling between their sites (expressed as free energy) is less than 50 calorie mol<sup>-1</sup>.

Seen in terms of the domain model of albumin, cooperative phenomena are interesting because they provide a readily available model for studying information transfer between discrete parts of the same polypeptide chain. In a clinical context, these studies are important because they imply that natural ligands and common drugs do not necessarily interact with albumin independently or in a simple competitive sense. Since some drugs are strongly bound by albumin, binding is an important factor in dose levels. The recent results suggest that the free concentration of such drugs could be markedly altered by the dietary fluctuation of plasma fatty acids. It is to be hoped that some of the current speculations and the now huge body of pharmacological measurements connected with albumin can soon be integrated. □

products are continually required for differentiation away from mesothorax.) These mutants produce transformations in the entire compartment normally requiring the gene product.

P. A. Lawrence (MRC Laboratory of Molecular Biology, Cambridge) described evidence that some segments are further subdivided into anterior and posterior compartments; here the engrailed gene product is apparently required exclusively for the posterior cells. The gene product is essential for the proper development of the posterior compartments and for the precise delineation of the boundary between anterior and posterior cells. He described unpublished work of G. Struhl (MRC, Cambridge) on the proboscis which showed that it is divided into anterior and posterior compartments and also dependent on the engrailed gene in a homologous way to the thoracic segments.

Another mutant which can be viewed within the same conceptual framework is the mutant *transformer* in which XX genetic females are made into almost perfect males.

R. Nöthiger (University of Zurich) described experiments which demonstrated that all embryos contain two separate primordia, one for male and one for female genitalia, each sex containing one 'silent' primordium. By making large *transformer* clones in an otherwise wildtype female, Nöthiger was able to conjure up a complete male set of genitalia in an intact female; *transformer* clones in the female primordium produced loss of part of the female genitalia.

The picture emerging from these four talks is one of a series of 'master' genes, which are activated in particular places, and which control the development of precisely defined pieces of the insect.

At the cellular level, O. Siddiqui (Tata Institute, Bombay) has studied the development of the *Drosophila* nervous system in a range of chemosensory mutants some of which show temperature sensitive development of the sensory pathway. The development of the nervous system was also the subject of a talk by J. White (MRC, Cambridge) who has used mutants and laser microsurgery to investigate the pattern of cell lineage in the ventral nerve cord of nematodes. Removal of individual cells by laser revealed that inductive interactions can occur, and that the vulva precursor cells can regulate their proliferation to compensate for the experimental elimination of specific cells. One alternative to conventional genetic approaches to the study of development is the use of cell-free systems, two of which were described at the meeting. R. Laskey

## Genes in development

from Ron Laskey, Peter Lawrence and Eddy De Robertis

A conference on Developmental Genetics was convened by the Accademia Nazionale dei Lincei and held in Rome on October 31–November 1, 1977.

PARTICIPANTS in two days of talks held at the Accademia Lincei in Rome—the oldest scientific foundation in the modern world, tried to tackle the ancient problem of how eggs plus genes produce animals. It was symptomatic of the renewed interest in *Drosophila* that the whole of the first day was devoted to that fly. E. B. Lewis (Cal-Tech) revealed some exciting new results in the bithorax system, the complex genetic locus responsible in part for the segmented pattern of the larva and adult fly. The scanning electron microscope showed up some hitherto unremarked distinctions between the different segments in the newly hatched larva so that the segmental patterns of new mutations and deficiencies could be studied. Flies deficient for the whole of the bithorax

complex developed only as far as the first stage larva, which consisted entirely of mesothoracic segments. Further analysis of partial deficiencies and 'constitutive' mutants indicated a group of new genetic functions (to the right of *pbx*) which Lewis called *polythoracic* and which are apparently necessary for the normal differentiation of the abdominal segments—all regarded as structures which diverge from the archetypal mesothorax.

A. Garcia-Bellido (Madrid University) pursued the problem of how the genes of the bithorax system are switched on in particular places. In his view the bithorax system responds to positional information in the egg; a process which can be interfered with in at least three ways: (1) the positional information can be disturbed, for instance by ether treatment of the blastoderm-stage egg. This results in groups of cells developing faithfully into a portion of inappropriate segment; (2) the same phenotype can be produced by certain mutations (*Rgpbx*, *Rgbx*) in genes which might produce regulatory molecules involved in the response of the bithorax system to the positional information; (3) mutations in the bithorax system itself (whose

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(MRC, Cambridge) described a *Xenopus* egg system in which chromatin assembly takes place. Each egg can convert an amount of DNA equivalent to 6000 nuclei into regularly spaced nucleosomes in the absence of DNA synthesis. Laskey has found that all the materials required for nucleosome assembly cofractionate in a single particulate fraction, suggesting that histones are organised into a nucleosome precursor complex before they bind DNA.

G. Tocchini-Valentini (CNR, Rome) described a *Xenopus* oocyte system which elongates and terminates replicating chromatin. But neither he nor Laskey could find evidence for initiation of DNA replication *in vitro*, although it can apparently be seen in micro-injected eggs.

The fusion of genetic and molecular approaches to the study of development provided a stimulating two-day meeting. □

## Problems with pollen

from Peter D. Moore

PALYNOLOGISTS studying sediments of Quaternary origin have certain advantages over colleagues concerned with older materials for there is a higher likelihood that both the taxa and the plant communities with which they deal remain extant. There are exceptions, of course, such as the tundra communities which occupied low latitudes and low altitudes in the periglacial conditions obtaining at the close of the last glaciation. One cannot assume that tundra communities of modern high latitudes or high altitudes have the same composition. But generally the Quaternary palaeoecologist uses the present as a Rosetta stone by which to interpret the past.

Reconstruction of the past mosaic of plant communities on the basis of a subfossil assemblage of pollen grains is currently being greatly assisted by accumulating information concerning modern pollen rain from contemporary plant communities. It is remarkable that for many decades palynologists have been content to interpret their fossil data without such information. Fortunately, however, indications from most modern pollen rain studies have confirmed the broad correlation between vegetation and its pollen output which has always been assumed. The work of Lichti-Federovich and Ritchie (*Rev. Palaeobotan. Palynol.* 7, 297;

1968), for example, on surface lake sediments in central Canada, showed that the major forest belts of the region could be distinguished on the basis of their pollen spectra. Certain anomalies were evident, such as the transport of spruce pollen into the treeless tundra areas of the north, but there was generally an acceptable agreement between vegetation and pollen rain.

Such studies have been chiefly concerned with the major components of both vegetation and the accumulating pollen assemblage, but detailed interpretation of palaeodata may also depend on the presence of relatively small quantities of a taxon with narrow ecological or phytosociological limits. It is often possible to infer the presence of certain vegetation types and hence certain habitats and microenvironments on the basis of the occurrence of these 'indicator types.' In this respect the work of Janssen has been of great value (for example *Ecol Monogr.* 37, 145; 1967). He has used the technique of comparing vegetation transects along environmental gradients with the pollen accumulating along them; in this way it is possible to observe how closely certain pollen types are associated with particular plant communities, even when their overall representation in the pollen spectrum is small.

On the basis of Janssen's work it is evident that pollen samples taken from the surface sediments of a water body will differ quite considerably from those in adjacent reed swamps or woodlands. So when interpreting fossil data, the nature of the sediment under consideration should be taken into account. One further aspect of this type of study which still lacks detailed documentation is the degree to which surface samples of the same type and under the same vegetation vary in their pollen composition. Is it possible that the variation within certain plant communities is greater than that between communities? In many surface pollen studies it has not been possible to glean this type of information because duplicated samples have been bulked before analysis, but a recent study of pollen rain in Alaska by Birks (*Can. J. Bot.* 55, 2367; 1977) now provides the necessary information for certain vegetation types.

Birks distinguished four major vegetation types, *Picea glauca* forest, which contained *Betula glandulosa* as a shrub layer, *Betula glandulosa* dominated shrub-tundra, *Populus balsamifera* stands within the forest, and open tundra vegetation dominated by *Dryas integrifolia*. In addition to the detailed description of these vegetation types, Birks collected samples of moss polsters, surface muds from lakes and superficial peat samples from sedge swamps from each of the plant com-

munities. A visual inspection of the data resulting from the analysis of these samples suggests that certain pollen characteristics are specifically associated with certain vegetation types. For example, only in samples originating from the *Populus*-dominated stands does the *Populus* pollen type occur at levels greater than about 2%, and only in the *Dryas* tundra does the level of *Dryas*-type pollen exceed about 2%.

Birks has subjected the pollen data to principal coordinates analysis, in which the pollen samples are ordinated with respect to one another on axes which represent dissimilarity. On one axis he shows a polarisation of the *Populus* forests and the *Dryas* tundra with the *Picea* forests and shrub-tundra forming an undistinguishable group in the centre. On the other axis the extremes are occupied by swamp peat samples and moss polsters respectively. One of the most important criteria which may account for this latter separation is the relative proportion of Cyperaceae pollen in the samples. One must conclude from this analysis that the degree of variation found within both the *Picea* forest and the shrub-tundra samples are such that the two vegetation types cannot be separated on the basis of their pollen spectra. This being so, one would not be able to differentiate between them in subfossil pollen assemblages.

This analysis was based only on those pollen types having a relative abundance of 5% or more of the total pollen sum in at least one of the samples; in other words, only the major pollen types are used for differentiation. There remains the possibility that indicator types of lower abundance could be used. Inspection of the pollen data suggests that this is unlikely to be a particularly helpful approach. Only one terrestrial herb taxon, *Astragalus*, is found in a single *Picea* forest sample but in none of the shrub tundra samples. On the other hand there are ten such taxa found scattered among the shrub-tundra samples which are not found in the spruce forest sites. As a final straw at which to clutch, one could compare the overall herb pollen diversity in the two sets of samples. Inspection indicates that the shrub-tundra has a generally larger component of low abundance, herbaceous taxa, possibly reflecting the open vegetation. A comparison of the two sets of data using a diversity index such as the Shannon-Wiener function might provide a means of resolving these two communities on the basis of their pollen spectra. Meanwhile, the fact that such problems can arise in the separation of modern vegetation types should encourage caution among those who seek to interpret fossil assemblages. □

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# review article

## Applications of proportional chambers and drift chambers in high-energy physics and other fields

G. Charpak\*

*Proportional counters, which were superseded in high-energy physics by developments such as spark and bubble chambers, have recently undergone modifications which have considerably increased their space and time resolution. These modified counters are finding applications not only in high-energy and nuclear physics but also in biology and medicine.*

PROPORTIONAL counters were an essential tool in nuclear physics until the beginning of the 1950s. Because of their practical importance their properties were extensively investigated and to a large extent well understood<sup>1</sup>. The powerful properties of structures different from the simple cylindrical shape were overlooked, however, and as we shall see, the multiwire structures permit space and time resolutions that are orders of magnitude better than those of cylindrical structures.

The counters were used for many purposes: counting of ionising radiations, measurement of their energy, localisation with an accuracy, both in space and time, usually set by the counter size. They have played only a minor part in the development of nuclear or sub-nuclear physics in the last quarter of a century, when they were superseded by the scintillation counters, faster and more versatile, the nuclear emulsions, the spark chambers of various types localising the position with accuracies better than a millimetre; the bubble chambers, visualising spatial configurations in particle reactions of an incredible complexity, and many other detectors.

A recent development<sup>2</sup>, based essentially on the same principles as the cylindrical counter but making a proper use of multiwire structures, is, however, making a radical change in the experimental strategies in low-energy and high-energy physics, and has also begun to find applications in several other fields, such as crystallography and medicine.

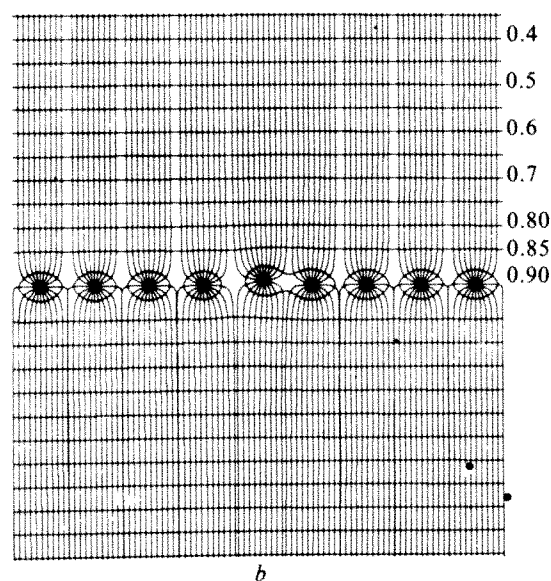
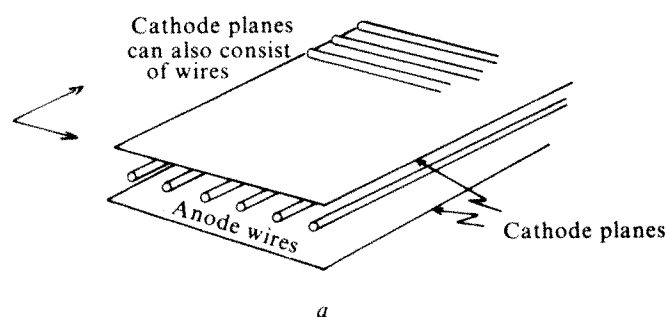
### Ordinary cylindrical proportional counters

Since this basic instrument is described in many text books, let us mention only its most salient features. It is typically made of a cylindrical cathode with a thin wire anode at its axis. The gas filling usually consists of a noble gas mixed with organic molecules. In such a structure the electrical field decreases as the inverse of the distance  $r$  to the axis. In the region close to the wire, the field is so intense that free electrons acquire enough energy in a mean-free path to ionise the molecules of the gas, thus leading to an avalanche of electrons localised close to the surface of the wire. A typical collision mean-free path at atmospheric pressure is  $1\ \mu\text{m}$ . The maximum size of an avalanche is around  $10^6$  electrons. For gains exceeding this limit, dependent on the gas and geometry, the ultraviolet photons emitted by the excited atoms induce a propagation of the discharge. The radial extension of an avalanche at atmospheric pressure is thus close to  $20\ \mu\text{m}$  only.

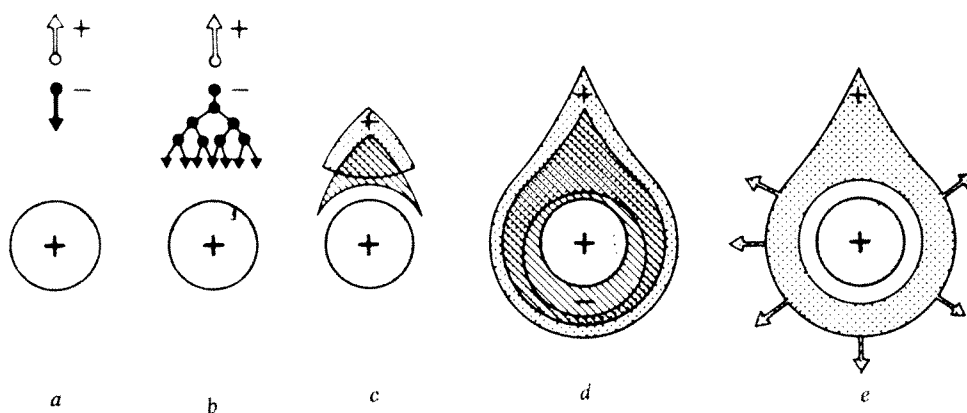
Since the process of amplification leading to the detectable pulse is produced after the ionisation electrons have reached the region close to the anode, the time of appearance of the pulse will depend on the initial position of the electrons, and will vary with the position of

the particle. This introduces an uncontrolled jitter in the timing of the particles, which reaches a few hundred ns for 1 cm diameter. This is one of the reasons why these counters were superseded by the scintillation counters, which, although more expensive, have a 100 times better resolution.

**Fig. 1** A multiwire proportional chamber: *a*, schematic view showing the cathode planes closely spaced around anode wires; *b*, electric field equipotentials and field lines in a multiwire proportional chamber. The effect on the field of a small displacement of one wire is also shown.



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**Fig. 2** Time development of an avalanche in a proportional counter. A single primary electron proceeds towards the anode (a), in regions of increasingly high fields, experiencing ionising collisions (b); owing to the lateral diffusion and photoionisation, a drop-like avalanche (c), surrounding the wire, develops. Electrons are collected in a very short time (1 ns or so) (d), and a cloud of positive ions is left, slowly migrating towards the cathode (e). Whether the cloud surrounds the wire or not, depends on the nature of the gas.

We shall see that by giving up the cylindrical structure, two steps have been accomplished: both the time and position of the particles can be measured with a high accuracy.

### Multiwire proportional chambers (MWPC)

The electrical structure of the chamber comprises thin, parallel, and equally spaced positively charged wires, sandwiched between two cathode planes. Figure 1 shows the electric field potentials in such a structure. We see that in the region close to the wire the electrical field is exactly the same as in a cylindrical structure. In other words, as soon as an electron liberated in the gas by an ionising radiation has reached the region of multiplication, it proceeds exactly as in a cylindrical counter. Although the wires are independent for the multiplication process, one may wonder whether their capacitive coupling does not short-circuit them and thus reduce them to a single electrode when they are as close as 1 mm. Indeed, when an external fast pulse is fed to one wire we observe the propagation of the pulse with the same sign, from wire to wire. This is not the case, however, with a pulse generated by the amplification process, and here we have to look into the detail of the pulse mechanism to

understand some of the properties which are responsible for the success of this type of structure.

Figure 2 shows the time development of the avalanche started by a single electron around a wire. Within a time shorter than a nanosecond, the avalanche results in a thin cloud of positive ions close to the wire, usually in a non uniform way. It was at first believed that the avalanches surround the wire. More recent experiments show that this is not usually the case and that this hypothesis is not required to explain the positive pulses on all electrodes neighbouring a sensing wire.

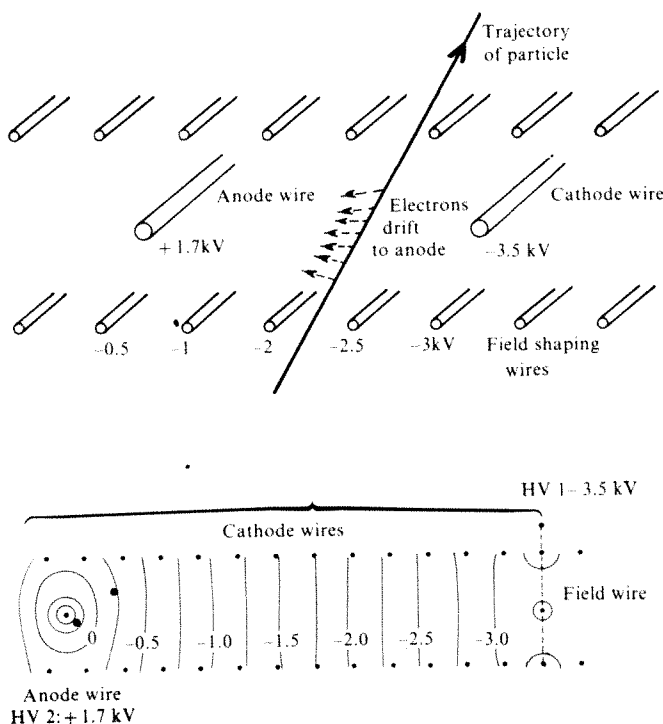
It is important to realise that the collection of the electrons does not usually give rise to a detectable pulse: it is too fast and too small. Its size is proportional to the potential drop experienced by the electrons while travelling in the gas, which is negligible since they travelled only a few  $\mu\text{m}$ . It is the motion of the positive ions that is responsible for pulses with the following characteristics. (1) Pulses are negative in the wires but positive in all surrounding electrodes. This is one essential feature: the neighbouring wires detect a positive pulse. Thus, the active wire is easily distinguished from its neighbours. It suffices to use amplifiers that are sensitive to one sign. (2) The pulses are fast-rising. The field is so intense close to the wire, usually above  $10^5 \text{ V cm}^{-1}$ , that rise-times of the order of 10 ns are observed at the beginning of the pulse, which then slows down and lasts for  $\mu\text{s}$ .

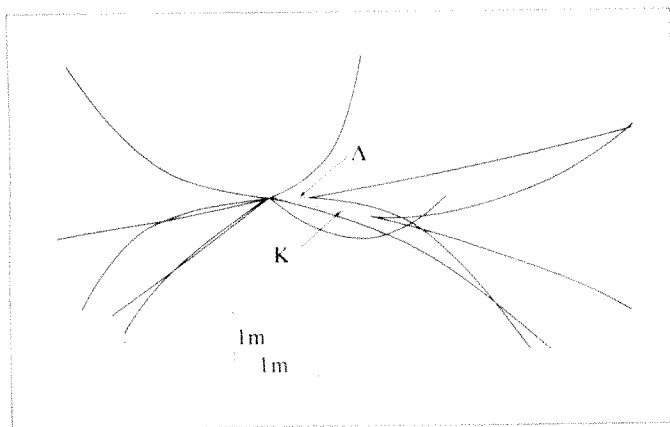
The important properties of a MWPC are as follows. (1) The space resolution is apparently dependent on the wire spacing since every wire behaves as an independent counter. In practice, 1 mm wire spacing is common for small structures (20 cm), 2 mm for large structures (200 cm), and chambers of  $5 \times 5 \text{ m}^2$  are being operated with 3 mm wire distance. (2) The space resolution of a chamber can be much better than the wire spacing. The coordinate of the avalanches along the wire can be measured by different methods. Accuracies better than  $100 \mu\text{m}$  can be obtained. Since a two-dimensional read-out is essential for the detection of neutral radiations, I shall discuss the recent progress in such measuring methods in connection with some applications. (3) The time resolution is also dependent on the wire distance. The closer the wire spacing the shorter is the time taken by the electrons liberated in the gas by an ionising particle to find a nearby wire to be detected. Practically 30 ns resolution is obtained with 2 mm wire spacing. (4) The maximum counting rate is limited locally by the space charge created by the positive ions. The chambers show a loss of gain at rates of the order of  $10^4 \text{ events s}^{-1} \text{ mm}^{-2}$ . Rates of several million counts  $\text{s}^{-1}$  are common in the practical use of the chambers.

### Drift chambers

The time lag between the appearance of a pulse and the distance to the wire of the ionising track can be used to measure this distance<sup>2,3</sup>. Typically it takes 200 ns for an electron to drift 1 cm in the chamber. This has given rise to an important class of detectors: the drift chambers. Measuring the time is very simple. Chambers with very large wire spacings can be used to make economical, large-size detectors. Figure 3 shows the structure of such a chamber. One example is given by the chambers built at Daresbury for an experiment at CERN: size 3.5 m, wire distance 12 cm, accuracy better than  $350 \mu\text{m}$  throughout the active area<sup>4</sup>. The intrinsic

**Fig. 3** Principle of construction of the adjustable field multiwire drift chambers: a, schematic view; b, equipotential lines. Cathode wires are connected to uniformly decreasing potentials, starting from ground in front of the anode. Field wires reinforce the field in the transition region to the next cell.





**Fig. 4** Computer display of a high-energy interaction viewed by a system of multiwire chambers. At an intersection of the CERN proton storage rings, a system of 70,000 wires in a magnetic field detects the coordinates of the particles from one interaction. Typical rates: 150,000 interactions s. Background:  $10^6 \text{ s}^{-1}$  chamber, resolution time  $\sim 100 \text{ ns}$ .

accuracy is of the order of  $50 \mu\text{m}$ , as shown in smaller chambers used in experiments<sup>3</sup>.

### Applications of MWPC and drift chambers

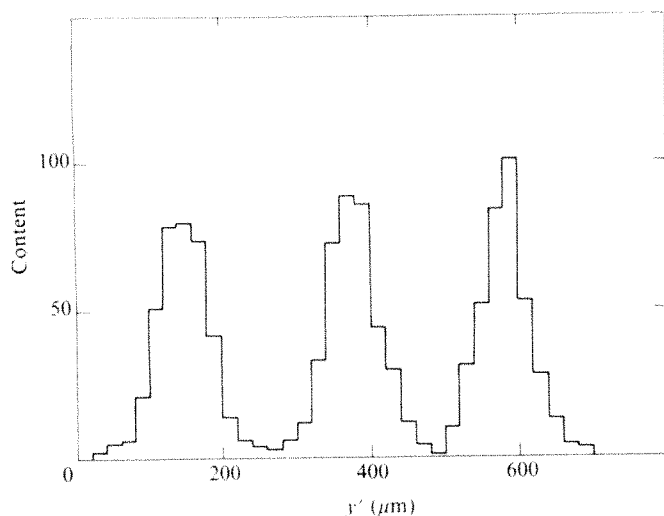
MWPC and drift chambers are now routine instruments in high-energy physics. They have in most cases replaced the spark chambers and sometimes the scintillation hodoscopes. They permit the detection of very rare events, for instance, in the hunting of new particles immersed in an enormous background of common reactions. The high time resolution and space resolution are the essential properties for this research.

Very large systems are in operation. Typical in this respect is the large detector equipping one intersect of the CERN storage rings. Set in a gap of about 10 m length, 1 m height, and 2 m width, chambers with wires of 1 m or 2 m length detect the trajectories of the particles produced in the head-on collisions of two 30 GeV protons. Figure 4 shows a complex event reconstructed by a computer from the data collected by 70,000 wires, 2 mm apart.

Many bubble chamber systems now use external systems of MWPC to give supplementary data on the particles emerging from the chambers.

Chambers are also now commonly used as detectors in nuclear physics in the focal plane of magnetic spectrometers, where

**Fig. 5** Accuracy along the wire. Histograms of the coordinates of the charge centroid along a wire for three positions of a collimated beam with  $200 \mu\text{m}$  separation (ref. 6).



accuracies of a fraction of a millimetre over lengths of the order of 1 m are easy to attain. Typical in this respect is the detector developed at the Massachusetts Institute of Technology<sup>5</sup>, where an accuracy of  $130 \mu\text{m}$  ( $2\sigma$ ) and an angular resolution of  $17 \text{ mrad}$  ( $2\sigma$ ) is obtained for relativistic electrons in the focal plane of a spectrometer.

Outside physics, MWPC are now being applied to problems of localisation of X rays and neutrons in medicine. These radiations produce in the gas low-range ionising secondaries which do not emerge from the chambers. It is essential to localise the coordinates of the avalanches from a single gap. Since the very beginning of the MWPC, the groups working in the field of neutral radiations have designed various analogue methods to measure the coordinates of avalanches along the wires<sup>2,6</sup>. They are based on the propagation of the signals in delay lines or resistive electrodes. A more recent development<sup>7</sup> illustrates the considerable accuracy that can be reached both along the wire and in the interpolation between wires. It is based on the measurement of the centroid of the pulses induced on cathode strips. Figure 5 shows the positions of three 1.5 keV X-ray beams, spaced about  $200 \mu\text{m}$  apart along the wire. A resolution of  $\sigma = 35 \mu\text{m}$  is obtained, of which only  $7 \mu\text{m}$  is contributed by the measurement, the remainder corresponding to the physical size of the charge distribution. The surprising thing is that this method gives also the position of punctual charge distributions, characteristic of X-ray secondaries, between the wires. It was for a long time postulated that the accuracy of a chamber is limited by the wire spacing. This is wrong in two cases: if the charges are distributed among several wires, the centroid method will give the interpolation; and if the charges are punctual, the centroid method will give the azimuth of the avalanche on the wire with an accuracy of about  $5^\circ$  for 2 mm wire spacings. This is illustrated by the radiograph of the word CERN<sup>7</sup>, cut out in a copper mask with letters of  $50 \mu\text{m}$  width, 1.5 mm height. Although the wire distance is 2 mm, a continuous response is obtained with all the avalanches occurring on the wire centred on the letters only (Fig. 6).



**Fig. 6** Radiograph of the word CERN. Dimensions of the word: 1.5 mm  $\times$  4 mm. Avalanches on only one wire: 2 mm wire spacing<sup>7</sup>.

This method will certainly be widely used in all problems where the two-dimensional read-out is important.

The most promising application of the chambers so far seems to be in the detection of X rays<sup>8,9</sup> or neutrons<sup>10,11</sup> diffracted by crystals made of large molecules, or by organic tissues with a regular structure<sup>12</sup>.

At CERN, a spherical drift chamber has been developed<sup>13</sup> to be used with the synchrotron radiation with the Orsay electron storage rings. Its present characteristics are: an angular aperture of  $90^\circ$ , an angular accuracy of  $2.5 \text{ mrad}$ , an efficiency of 100% for 8 keV photons, and a possible ultimate rate of  $10^6 \text{ s}^{-1}$ . A gain of at least 1,000 in the speed of data-taking is expected with respect to the conventional detection methods used with diffractometers. This opens up new fields of research, for instance in the study of quickly evolving structures.

Nuclear medicine also has problems of imaging, chiefly in the detection of X rays that are much more energetic. The minimum is the radiation of 27 keV emitted by  $^{125}\text{I}$ , commonly used in labelling the thyroid tissues. The main interest, however, lies in radiations well above 100 keV, where the chambers are quite inferior to the widely used scintillation cameras in two respects: the low efficiency, and the poor spatial resolution due to the large range of the



secondary electrons in the gas. So far this has limited the applications to fields where radiations below 100 keV are of interest. Typical in this respect is the work undertaken at Rutherford Laboratory<sup>14</sup>, where a chamber measures bone densities with the 42 keV X rays emitted by <sup>253</sup>Gd. The localisation of the X rays can be better than with NaI cameras<sup>15</sup> and they cost far less.

A possible way of overcoming the low density of the gas is to use a drift space filled with suitable converters. Several groups<sup>16,17</sup> are at present actively developing this approach. Efficiencies of up to 15% with accuracies of a few millimetres can be reached at 500 keV.

These chambers suffer from two drawbacks: no energy resolution, and a poor resolution time close to 0.5  $\mu$ s. They find a possible important application for positron cameras. The correlation of the two annihilation  $\gamma$ -rays give such a good selection of non-scattered  $\gamma$ -rays that it compensates partially for the lack of energy resolution. The poor time resolution may be a fatal obstacle to the use of this technique for medical applications. Present positron cameras based on NaI counters can operate with 10-ns resolution times. They lead to such expensive instruments, however, and the technique of positron imaging could be so powerful with good, cheaper detectors, that it is still worth making a thorough investigation of the possibilities offered by MWPC, which are better suited to large size, low cost detector systems than are the NaI cameras.

## Conclusions

Multiwire chambers and drift chambers offer an interesting example of a technology developed in high-energy physics laboratories with a fair chance to find important applications in fields remote from high-energy physics. Whenever methods require the measurement of the position of ionising radiation, a possible application of these detectors can be found; this offers a good opportunity to make use of the huge effort in capital and skills invested by experimental groups working in high-energy physics.

1. Curran, S. C. & Craggs, J. D. *Counting Tubes* (Butterworths, London 1950).
2. Charpak, G. *A. Rev. nucl. Sci.* **20**, 195 (1970).
3. Sauli, F. *Principle of operation of multiwire proportional and drift chambers* CERN 77-09 (1977).
4. Connel, K. A. *et al.* Preprint Daresbury Laboratory DL 272.
5. Bertozzi, W. *et al.* *Nucl. Instrum. Meth.* **141**, 457 (1977).
6. Rice-Evans, P. *Spark, Streamer, Proportional and Drift Chambers* (Richieu, London, 1974).
7. Charpak, G., Petersen, G., Policarpo, A. & Sauli, F. *Nucl. Instrum. Meth.* (in the press).
8. Perez-Mendez, V. LBL-3851 (Lawrence Berkeley Laboratory, 1975).
9. Cork, C. *et al.* *J. appl. Cryst.* **7**, 319-323 (1974).
10. Alberi, J. *et al.* *Nucl. Instrum. Meth.* **127**, 507 (1975).
11. Allemann, R. *et al.* *Nucl. Instrum. Meth.* **126**, 29 (1975).
12. Faruqi, A. R. *IEEE Trans. nucl. Sci.* **NS 22**, 2066 (1975).
13. Charpak, G. *et al.* *Nucl. Instrum. Meth.* **122**, 1307 (1974).
14. Reading, D.-H. *Rutherford Lab. Rep.* RL 76-047 (1976).
15. Bateman, J. E., Walters, M. W. & Jones, R. E. *Nucl. Instrum. Meth.* **135**, 235 (1976).
16. Chu, D. *et al.* LBL 5116 (1976).
17. Jeavons, A. P. & Cate, C. *IEEE Trans. nucl. Sci.* **NS 23**, 640 (1976).

# articles

## Past volcanism revealed by Greenland Ice Sheet impurities

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*Past volcanic aerosols have left Greenland Ice Sheet layers with elevated specific electrical conductivities that can often be used to estimate the magnitude of individual eruptions. Records of past volcanism may, therefore, be extended and help solve the problem of possible climatic correlation.*

PRECIPITATION falling on the two large ice sheets in Antarctica and Greenland is extremely pure<sup>1,2</sup>. The low background impurity level makes polar glacier ice suitable for investigating past violent volcanism in the form of additional impurities deposited shortly after the eruptions and stored in the ice.

Disregarding gases and organic material<sup>3</sup>, the deposition of impurities on the Greenland Ice Sheet is of the order of 50 kg km<sup>-2</sup> yr<sup>-1</sup> in times of low volcanic activity<sup>4</sup>. Assuming that the mean aerosol residence time in the upper troposphere is approximately 1 month<sup>5</sup>, the above figure corresponds to a background aerosol load of the order of 10<sup>9</sup> kg in the upper northern hemisphere troposphere, which is close to an estimate based on Mauna Loa data<sup>6</sup>. Aerosols of volcanic origin may be detectable as increased aerosol fall-out, if their contribution to the upper tropospheric aerosol load is significant relative to the above mentioned background.

Violent volcanic eruptions inject into the atmosphere large quantities of impurities, part of which significantly reduces the

atmospheric transmissivity on a global or hemispheric scale up to three years after the eruptions. For example, the Krakatau (1883) and the Katmai (1912) eruptions caused a 20% reduction of the Northern Hemisphere atmospheric transmissivity by each injecting 16  $\times$  10<sup>9</sup> kg aerosol material into the northern stratosphere<sup>6</sup> (Krakatau probably injected a similar amount into the southern stratosphere as well). Assuming that this material is deposited within 1 or 2 yr, the average volcanic contribution to the total upper northern tropospheric aerosol load in these periods was probably of the same order as the non-volcanic background (10<sup>9</sup> kg).

Therefore, in spite of the spreading of the eruption cloud and the complicated deposition pattern caused by non-uniform wash-out by precipitation, any 'clean-air' location must be expected to receive significantly elevated amounts of aerosol fall-out in periods of high volcanic activity, at least in the same hemisphere. The most favourable areas for tracing past volcanism are the high altitude regions of the two large ice sheets, where the background concentration of non-volcanic aerosols is low. The Greenland Ice Sheet has the additional advantage of being fed by relatively frequent, heavy snow falls. The impurity concentration in the snow is proportional to the temporary aerosol load in the air<sup>7</sup> and since the snow is accumulated with little melting and no run-off, analyses of absolute-dated Greenland cores provide a record of atmospheric turbidity, essentially above ~ 2,000 m altitude.

None of the impurity nor its constituents has so far been shown

to be a specific volcanic tracer. Previous analyses<sup>8</sup> performed on an ice core from Dye 3 (Fig. 1), indicated that most solid micro-particles above  $0.4\text{ }\mu\text{m}$  radius are continental surface dust, even during periods of high volcanic activity. But the high sulphate content in the stratosphere shortly after recent violent eruptions suggests that most of the volcanic material in the stratosphere consists of sulphate, originating from the release of  $\text{H}_2\text{S}$  and  $\text{SO}_2$  (ref. 9). Hence, elevated concentrations of  $\text{H}_2\text{SO}_4$  and/or  $(\text{NH}_4)_2\text{SO}_4$  might be detectable in Greenland snow up to three years after the volcanic event. Impurities, particularly of sulphuric acid, greatly influence the specific electrical conductivity,  $\sigma$ , of the melted ice, and as we analysed thousands of samples, this simple parameter was chosen as an indicator of volcanic activity. Of course,  $\sigma$  profiles cannot replace the comprehensive chemical analysis program that has commenced<sup>10</sup>, but they do reveal important characteristics of volcanic fall-out and may be used as a simple means of showing which of the numerous core increments are promising objects for detailed geochemical analyses.

### Volcanic impurity concentration index

Using  $\sigma$  as a volcanic activity indicator implies a comparison of  $\sigma$ -profiles along absolute-dated ice cores with historical records of the volcanic dust production. But such records based on objective measurements only span the past 100 years. Extending the comparison beyond this period, therefore, necessitates using records based on indirect evidence, such as the total amount of material ejected. In assessing the relative magnitude of the eruptions as dust producers, Lamb<sup>11</sup> includes historical accounts of (non-instrumental) observations of optical phenomena in the upper atmosphere. Lamb's volcanic dust production index (v.d.p.i.) is shown in Fig. 2a dating back to 1770. There is only one adjustment: the v.d.p.i. for the Shtyubelya Sopka eruption at  $52^\circ\text{N}$  in 1907 (equal to that of Katmai, 1912) has been reduced by a factor of 5 in view of radiation measurements in the  $30\text{--}60^\circ$  northern latitude belt<sup>12</sup>. The open bars are mainly derived from an assumed relationship between climatic temperature anomalies and atmospheric dust veils.

In order to estimate the volcanic ion concentration in Greenland precipitation, the v.d.p.i. has to be corrected to account for the residence time of the aerosols in the atmosphere and the non-uniform distribution pattern of the fall-out. The correction has been made by accounting for (1) a temporal distribution of the fall-out corresponding to a residence time of 1 yr for aerosols in the high latitude stratosphere (2) a time lag of 1 yr for the high northern latitude fall-out of volcanic debris originating from eruptions south of  $50^\circ\text{N}$ , and (3) spreading of the eruption cloud on its travel to high northern latitudes by using a correction factor  $F$  on the v.d.p.i. of all eruptions. For simplicity, and with a view to fall-out data for fission products<sup>13,14</sup>, supplemented by total  $\beta$ -activity data from the polar ice sheets<sup>15,16</sup>, the  $F$ -factor is equal to 1 for latitudes of eruptions north of  $50^\circ\text{N}$ , 0.5 in the  $20^\circ\text{--}50^\circ\text{N}$  latitude belt, 0.2 in the  $20^\circ\text{S}$  to  $20^\circ\text{N}$  belt and 0.07 for eruptions south of  $20^\circ\text{S}$ .

As precipitation contains a representative sample of the aerosols in the precipitating air mass, the procedure described above leads to a new index (Fig. 2b), which may be called a volcanic impurity concentration index (v.i.c.i.) for high northern latitude precipitation.

### Material and techniques

Specific conductivity profiles have been measured along three ice cores recovered from North (Hans Tavsén), Mid (Crête) and South (Dye 3) Greenland (Fig. 1), all recovered under the Greenland Ice Sheet Program. The Crête  $\sigma$  profile is continuous through the period AD 1770–1972. The Crête and Dye 3 cores are absolute-dated<sup>16</sup>, whereas the Hans Tavsén core is dated by ice flow model considerations back to 1815 and from then on by counting annual cycles in the micro-particle concentration<sup>16</sup>.

The cores were sampled in sequence averaging four samples for each annual layer as revealed by seasonal cycles in heavy oxygen-isotope concentration<sup>16</sup>. The individual samples were cut from the cores at  $-12^\circ\text{C}$  in a clean air bench after removal of a more

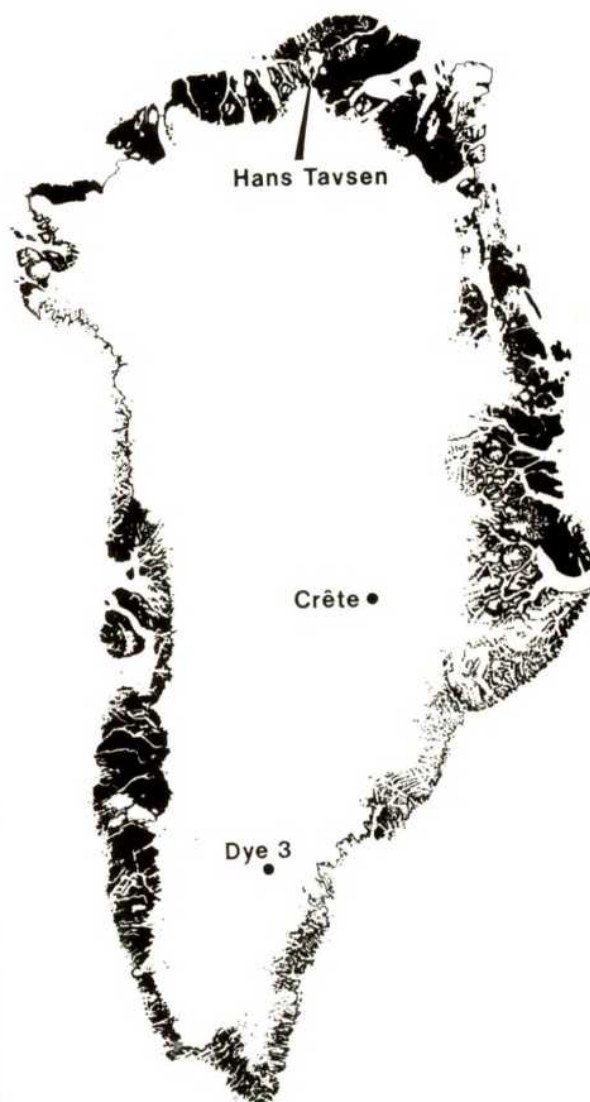


Fig. 1 The Greenland Ice Sheet with the drill sites, where the investigated ice cores were recovered: Dye 3 (elevation  $e = 2,480\text{ m}$ ; snow accumulation rate  $a = 49\text{ g cm}^{-2}\text{ yr}^{-1}$ ); Crête ( $e = 3,170\text{ m}$ ;  $a = 25\text{ g cm}^{-2}\text{ yr}^{-1}$ ); and Hans Tavsén ( $e = 1,200\text{ m}$ ;  $a = 17\text{ g cm}^{-2}\text{ yr}^{-1}$ ).

than 5 mm surface layer that contains essentially all of the relevant contaminants in an undamaged core. Occasional cracks and other regions of possible deep contamination were avoided. The ice samples were melted in clean plastic beakers, and the solid micro-particle concentration including particles down to  $0.05\text{ }\mu\text{m}$  radius, was measured by a light scattering method<sup>8</sup> and also in many cases by the standard Coulter counting method. Often the pH was measured by standard procedures. The conductivity measurements required larger amounts of water. In order to save core material, it was necessary to dilute most of the samples up to three times with deionised, redistilled water ( $\text{pH} = 5.6$ , specific conductivity  $\sigma = 130\text{ }\mu\text{S m}^{-1}$ ). This introduced some false noise in the low  $\sigma$  values, and perhaps up to a 15% increase in the  $\sigma$  background. The mixtures were allowed to saturate with the carbon dioxide in the ambient air at  $25^\circ\text{C}$ , which changed  $\sigma$  by less than 10%, because both the ice<sup>17</sup> and the dilute were near saturation beforehand. Finally,  $\sigma$  was determined by standard procedure.

### Results and discussion

The annual mean solid micro-particle mass concentration at Crête, determined by total number and size distribution analyses, is plotted in  $\mu\text{g per kg}$  of ice in Fig. 2d. There is no significant correlation with the v.i.c.i. in section b, which indicates that

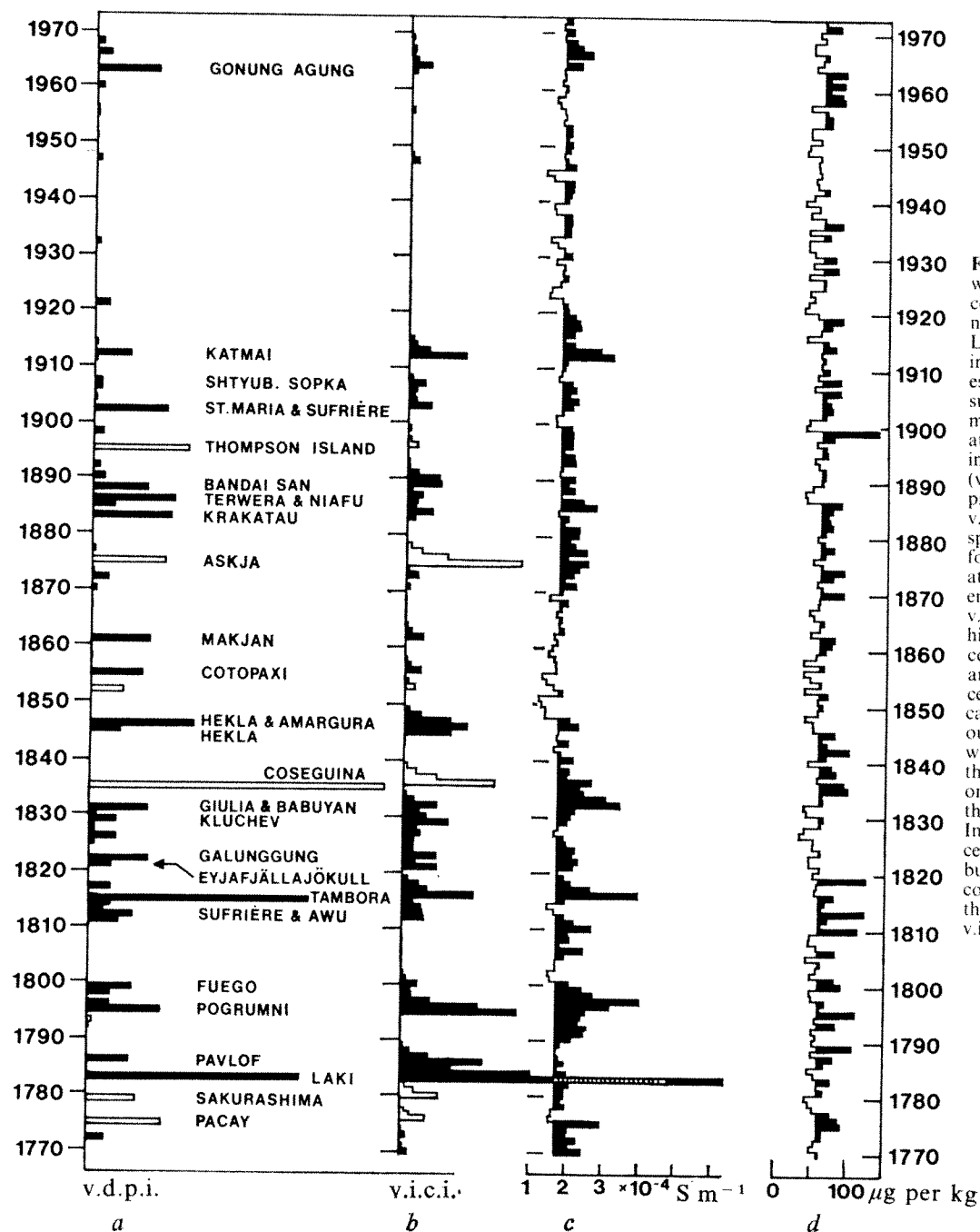


Fig. 2 Volcanic activity compared with impurity profiles along an ice core from Crête, Greenland, spanning the period 1770-1972. *a*, Lamb's volcanic dust production index (v.d.p.i.); open bars refer to estimates mainly based on an assumed relationship between climatic temperature anomalies and atmospheric dust veils. *b*, Volcanic impurity concentration index (v.i.c.i.) for high northern latitude precipitation, derived from the v.d.p.i. by correction for latitudinal spreading of the eruption cloud and for residence time of aerosols in the atmosphere. The Icelandic Laki eruption, 1783, stands out with a v.i.c.i. more than twice the second highest v.i.c.i. (1795). *c*, Specific conductivity of melted samples of annual precipitation. Values exceeding the mean in the volcanically quiet period 1920-60 are set out in black. Significant correlation with the v.i.c.i. shows that part of the soluble impurities of volcanic origin. The value for 1783 is more than twice the second highest. *d*, Insoluble micro-particle mass concentration (in  $\mu\text{g per kg of ice}$ ). The bulk of insoluble impurities must be continental surface dust, because the curve has no correlation to the v.i.c.i., and no increasing trend in the industrial period.

essentially all of this fallout at Crête is of continental surface origin. Furthermore, the lack of significant increase in the upper part of curve *d* shows that little, if any, industrial solid dust reaches Central Greenland.

According to Fig. 2b the Icelandic Laki eruption in 1783 (historically the greatest lava extrusion, some  $12 \text{ km}^3$ , on Earth) was presumably responsible for the highest fall-out of volcanic debris in Greenland in the past 200 years, which suggests the 1783 ice layer as favourable for a pilot test. Figure 3 shows  $\sigma$  profiles through the annual layers deposited in the period 1780-86. The shaded part of the  $\sigma$ s represents the contribution of  $\text{H}^+$ ,  $\sigma_{\text{H}^+}$ , calculated from the pH data and corrected for  $\text{CO}_2$  induced ions. The Laki eruption is revealed in the Dye-3 and Crête cores by elevated  $\sigma$  and  $\sigma_{\text{H}^+}$  values in the layer previously absolute-dated at 1783. In the Hans Tavsén core the peak values appear in a layer dated at 1784 mainly by the less accurate ice flow model calculations. The Hans Tavsén peaks are bipartite due to a layer of refrozen meltwater that has percolated from a younger summer layer. At this station, located on a medium altitude, separate ice sheet, and only some 15 km from snow-free areas, the ice chemistry is complicated by the dust content being more than an

order of magnitude higher than at the other stations. The  $\sigma_{\text{H}^+}$  shows that melted Hans Tavsén precipitation has a strong acid reaction only in periods of heavy volcanic fall-out.

In Fig. 2c the annual mean  $\sigma$  values at Crête are plotted over the period 1770-1972. Those exceeding the 40 yr mean  $\sigma$  value ( $170 \mu\text{S m}^{-1}$ ) for the volcanically quiet period 1920-60 are set out in black. The variability of  $\sigma$  in this period should be considered as noise, and deviations of the same order in the rest of the curve are therefore insignificant. Nevertheless, the small rise in  $\sigma$  in the late 1910s may be significant, because the HCl and HF production in 'the valley of 10,000 smokes' created by the Katmai eruption has been estimated<sup>18</sup> at  $1.4 \times 10^9 \text{ kg yr}^{-1}$  up to 1920.

Sudden rises in v.i.c.i. and  $\sigma$  generally occur simultaneously or with a short time lag in  $\sigma$ . As for eruptions south of  $50^\circ\text{N}$  any time lag in  $\sigma$  between -1 and +1 year may occur, in view of the general 1 yr shift introduced when transferring the Lamb's v.d.p.i. into the v.i.c.i. in Fig. 2b. Eruptions north of  $50^\circ\text{N}$  should be revealed by sudden increases in  $\sigma$  with time lags between zero and +1 yr, assuming the ice core time scale to be exact.

Although Lamb's v.d.p.i. (Fig. 2a) is not specifically designed to represent the volcanic production of gases and ionic com-



**Table 1** Background composition of impurities in melted Greenland glacier ice

Element	Concentration ( $\mu\text{mol kg}^{-1}$ )	Location	Ref.
H	3.5	Crête	This work
Na	1.0 average	Dye 3, Crête	10
Si	1.4	135 km WSW of Crête	19
Al	0.3	300 km WSW of Crête	2
NH <sub>4</sub>	0.2	300 km WSW of Crête	2
	0.3	Crête	10
Ca	0.2	Dye 3	(Busenberg & Langway, personal communication)
SO <sub>4</sub>	0.8	Crête	10
Cl	1.6	Dye 3	20
		300 km WSW of Crête	2
		Dye 3	20

pounds, a statistical analysis of the v.i.c.i. and  $\sigma$  time series shows a highly significant correlation coefficient ( $R = 0.6$ ;  $P > 99.9\%$  for  $n = 20$  degrees of freedom). Furthermore, Fourier spectral analysis shows less than 1 yr time lag for any frequency higher than  $0.02 \text{ yr}^{-1}$ , the coherence being of the order of 0.6 for the same frequencies.

If, consequently, elevated  $\sigma$  values are accepted as indicative of fallout of volcanic acids produced in the northern hemisphere, Fig. 2c suggests that unnoticed eruptions of considerable magnitude took place around 1810. Furthermore, the v.d.p.i. for the Askja eruption, 1875 (and Pavlof, Alaska, 1786?) may have been overestimated, as an Icelandic eruption of the estimated magnitude would probably be revealed by more than a barely significant increase in  $\sigma$ .

Some of the available ice sheet chemistry data are presented in Table 1. They suggest a simple explanation of the  $170 \mu\text{S m}^{-1}$   $\sigma$  background at Crête being due mainly to the  $\text{H}^+$ ,  $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ . First, quite a few elements (such as, Si, Al and Mg) may be disregarded, because they are mainly present in solid micro-particles that do not contribute to  $\sigma$ . For example, using the Al content ( $5 \mu\text{g per kg ice}$ ) as an indicator of igneous rock material<sup>21</sup> gives an order of  $60 \mu\text{g}$  of insoluble material per kg of ice, in agreement with the overall mean value in Fig. 2d. The Ca concentration is seven times higher than in igneous rocks, but still insignificant for  $\sigma$ . Second,  $\text{CO}_3^{2-}$  can be disregarded, because at Crête the pH averages 5.46 in non-volcanic periods (lower in volcanic periods). The pH corresponds to a background  $\text{HCO}_3^-$  concentration  $[\text{HCO}_3^-]$  of  $1.3 \mu\text{mol kg}^{-1}$ , and  $[\text{H}^+]$  of  $3.5 \mu\text{mol kg}^{-1}$ .

The total anion concentration must of course be balanced by the total cation concentration:

$$[\text{H}^+] + [\text{Na}^+] + [\text{NH}_4^+] = 2[\text{SO}_4^{2-}] + [\text{Cl}^-] + [\text{HCO}_3^-].$$

The  $1.0 \mu\text{mol kg}^{-1}$   $[\text{Na}^+]$  originates from sea salts, which also accounts for the equivalent part of the  $[\text{Cl}^-]$ . Similarly, the  $0.3 \mu\text{mol kg}^{-1}$   $[\text{NH}_4^+]$  may be interpreted as being due to  $(\text{NH}_4)_2\text{SO}_4$  of continental origin, thus accounting for  $0.15 \mu\text{mol kg}^{-1}$   $[\text{SO}_4^{2-}]$ . Subtracting these concentrations, and using the values listed in Table 1, the equation shows an essential balance between the  $3.5 \mu\text{mol kg}^{-1}$   $[\text{H}^+]$  and  $0.8 - 0.15 = 0.65 \mu\text{mol kg}^{-1}$   $[\text{SO}_4^{2-}]$  plus  $0.6 \mu\text{mol kg}^{-1}$   $[\text{Cl}^-]$  plus  $1.27 \mu\text{mol kg}^{-1}$   $[\text{HCO}_3^-]$ , a total corresponding to  $3.2 \mu\text{mol kg}^{-1}$  monovalent anion. Other elements may be present in minor amounts, but according to the above,  $\text{H}_2\text{SO}_4$  contributes 33%,  $\text{CO}_2$ -induced ions 29%,  $\text{HCl}$  15%,  $\text{NaCl}$  8% and  $(\text{NH}_4)_2\text{SO}_4$  3%, a total of 89% of the  $\sigma$  background,  $170 \mu\text{S m}^{-1}$ , at Crête.

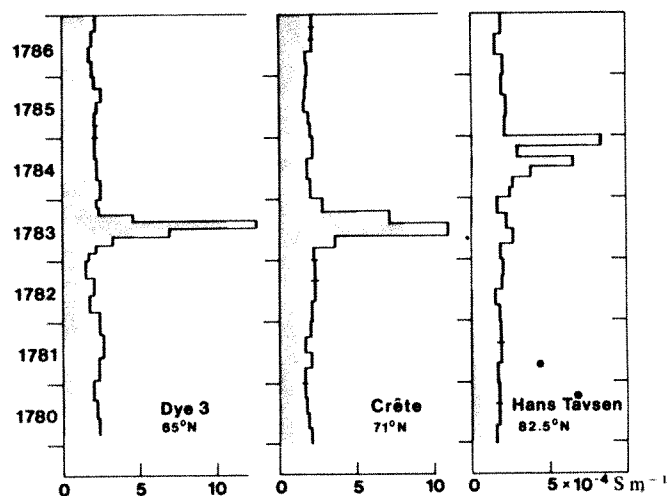
Looking at the Laki event as revealed in the Dye 3 ice (Fig. 3) the  $\sigma$  peak value (in part of the mid 1783 layer) is  $\sigma = 1,400 \mu\text{S m}^{-1}$ . The pH is low, corresponding to  $[\text{H}^+] = 33 \mu\text{mol kg}^{-1}$ , and the  $[\text{SO}_4^{2-}]$  amounts to  $12 \mu\text{mol kg}^{-1}$ . Hence, in addition to  $\text{SO}_4^{2-}$ , other anions (probably  $\text{Cl}^-$ , but this is not important here) contribute  $33 - (2 \times 12) = 9 \mu\text{mol kg}^{-1}$ . These concentrations fully account for the measured  $\sigma$  peak value.

The lack of a recent increasing trend of the Crête  $\sigma$  curve (Fig. 2c) suggests that industrial sulphates do not reach Mid Greenland

in appreciable amounts. Other authors<sup>20,22</sup> have drawn the opposite conclusion from North and South Greenland data, showing sulphate concentrations in the period 1964–71 that are roughly twice the concentration in pre-industrial periods. The  $\sigma$  curve in Fig. 2c suggests however, that the Agung eruption 1963 may be responsible for the elevated sulphate (and chlorine) values in the late 1960s, although detailed chemical analyses of a longer and continuous part of the ice cores are needed for firm evidence.

The rise of the excess  $[\text{H}^+]$  in ice layers containing debris from a high northern latitude eruption can be used to roughly estimate the global  $\text{H}^+$  fall-out by applying the same latitudinal distribution pattern as in the case of  $^{90}\text{Sr}$  fall-out from high northern latitude bomb tests. In 1962, the global fall-out of  $^{90}\text{Sr}$  (mainly from the Soviet tests in 1961) was  $1.2 \text{ MCi}$  which was  $8.6 \times 10^8$  times the fall-out per  $\text{km}^2$  at Crête (H. B. Clausen, personal communication). Applying this factor to the fall-out of volcanic  $\text{H}^+$  per  $\text{km}^2$  in 1783 at Crête ( $2.4 \text{ kg km}^{-2}$ ) gives a total fall-out of  $2 \times 10^6$  tons  $\text{H}^+$  during the Laki eruption, or  $100 \times 10^9 \text{ kg}$  of mainly  $\text{H}_2\text{SO}_4$ , as shown in Table 2. Similar estimates can be made for eruptions south of  $50^\circ\text{N}$ , if the  $F$ -factor is accounted for. For example, the Tambora eruption in 1815 seems to have contributed no less than  $300 \times 10^9 \text{ kg}$  of acids to the stratospheric aerosol load. As to the few violent eruptions that have occurred within the period of instrumental observations, the Katmai eruption in 1912 is revealed by a change in  $[\text{H}^+]$  high enough to allow a fairly accurate estimate for comparison with independent estimates. A more detailed  $\sigma$  curve shows that the fall-out from the Katmai eruption lasted approximately 1.5 yr, and the total production of  $\text{H}_2\text{SO}_4 + \text{HX}$  is estimated at  $20 \times 10^9 \text{ kg}$ . This is close to Junge's estimate<sup>6</sup> of  $16 \times 10^9 \text{ kg}$  total mass, suggesting that most of the Katmai aerosols were acids. But, chemical

**Fig. 3** The Icelandic Laki eruption, 1783, revealed in the Dye 3, Crête and Hans Tavsén ice cores by peak values of specific conductivity in melted samples. The Hans Tavsén ice core is not dated absolutely, so the high conductivity layer may very well originate from 1783. The shaded areas show the contribution of hydrogen ions, not induced by  $\text{CO}_2$ .





**Table 2** Estimates of global fall-out of volcanic acids based on pH analyses on the Crête ice core

Eruption	Duration of deposition of debris yr	Precipitation during period of deposition kg km <sup>2</sup> × 10 <sup>8</sup>	Average rise in (H) through deposition μ mol kg <sup>-1</sup>	Fall-out of volcanic H at Crête kg km <sup>2</sup>	F	Global fall-out		Other estimates Total mass 10 <sup>9</sup> kg	Ref.
						H <sup>+</sup> 10 <sup>9</sup> kg	H <sub>2</sub> SO <sub>4</sub> + HX 10 <sup>9</sup> kg		
Laki, 1783	0.75	2	12	2.4	1	2	100		
Tambora, 1815	1	2.5	5.2	1.3	0.2	6	300		
Krakatau, 1883	2	5	(0.4)	(0.2)	0.2	(0.9)	(45)	32	(6)
Katmai, 1912	1.5	2.8	1.6	0.5	1	0.4	20	16	(6)

analyses are needed to reliably assess the magnitude of several medium sized low latitude eruptions (such as Agung, 1963) and to clarify the reasons for the instability of the background in the  $\sigma$  profile. Furthermore, in one case (the Aleutian eruptions 1795 and 1796), the volcanic fall-out had a pH as high as 5.53; consequently, the high  $\sigma$  in this period must be due to high concentrations of other components ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>Cl?) of relatively low equivalent conductances, which, of course, influences the estimation of the magnitude of these eruptions.

The possible influence of atmospheric turbidity on climate is complicated, because aerosols contribute to the albedo as well as the absorption of radiation. The net-effect upon global temperatures is poorly known. For comparison with climatic records, the  $\sigma$  profile is used to design an objective volcanic aerosol production index (by a procedure opposite to that used to design the v.i.c.i.) and turn it into an index of possible influence of volcanic aerosols upon northern hemisphere temperatures, with due account for the latitudes of the eruptions and for the residence time of the aerosols in the atmosphere. This index has no significant correlation to annual mean temperatures in England<sup>23</sup>, even with time lags up to 10 yr ( $R \sim -0.06$  to  $-0.19$ ;  $P \sim 30$  to  $70\%$ ;  $n = 20$ ), which cannot be due solely to the index being imperfect. The lack of correlation confirms the result of a similar attempt<sup>24</sup> using Lamb's dust veil index. When using 10-yr averages, one finds a high but insignificant correlation ( $R = -0.55$ ,  $P = 93\%$ ,  $n = 8$ ). This procedure is not advisable, because it may mask important time lags. For example, the cooling in the 1960s began several years before the Agung eruption 1963. Other authors<sup>25</sup> have claimed that volcanic aerosols have made important contributions to climatic changes in the past, but they only considered details of the 1884–1970 period that seems to include too few great volcanic events to justify a statistically significant statement.

In conclusion, specific conductivity profiles reveal violent volcanism in the past. Elevated conductivities, due to fall-out of acids, usually allow the contribution of the eruptions to the high altitude aerosol budget to be estimated if the locations of the eruptions are known. If they are unknown, comparison of conductivity profiles along ice cores recovered at different latitudes in Greenland and Antarctica may lead to estimates of the

volcanic activity beyond the range of historical records, at least 10,000 years ago, and particularly when combined with chemical analyses. Ice from the Wisconsin glaciation was deposited in a different climatic regime and contains high and strongly variable amounts of chemical elements<sup>4</sup>, which may complicate the interpretation. One way of using long volcanic activity records would be to check if they have any relation to climatic changes as revealed by stable isotope profiles along the same cores. Another aspect concerns the high concentrations of hydrogen ions in layers deposited in periods of high volcanic activity, which suggests anomalous dielectric properties of such ice. Series of layers containing high concentrations of volcanic impurities is, therefore, a possible explanation of the internal reflection layers encountered in radio-echo sounding<sup>26</sup>.

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- Langway, C. C., Jr. *Geol. Soc. Am., Spec. Pap.* **125**, 1–186 (1970).
- Herron, M. M., Weiss, H. V. & Langway, C. C., Jr. *Geochim. cosmochim. Acta* (in the press).
- Fredskild, B. & Wagner, P. *Boreas* **3**, 105–108 (1974).
- Cragin, J. H., Herron, M. M., Langway, C. C., Jr. & Klouda, G. *SCOR/SCAR, Polar Ocean Conf.* (McGill University, Montreal, Canada, 1974).
- Junge, C. E. in *Air Chemistry and Radioactivity* 1–382 (Academic, New York, 1963).
- Junge, C. E. *Proc. Int. Conf. Structure, Composition General Circulation of the Upper and Lower Atmosphere on Possible Anthropogenic Perturbations* **1**, 1–14 (1974).
- Junge, C. E. *Proc. IUGG Symp. Isotopes and Impurities in Snow and Ice* (in the press).
- Hammer, C. U. *Proc. IUGG Symp. Isotopes Impurities in Snow and Ice* (in the press).
- Castleman, A. W., Munkelwitz, H. R. & Manowitz, B. *Tellus* **26**, 222–234 (1974).
- Langway, C. C., Jr., Cragin, J. H. & Klouda, G. A. *Proc. IUGG Symp. Isotopes and Impurities in Snow and Ice* (in the press).
- Lamb, H. H. *Phil. Trans. R. Soc. Lond.* **266B** 425–539 (1970).
- Dyer, A. J. *Q. J. R. Met. Soc.* **100**, 563–571 (1974).
- Health and Safety Laboratory, *Fallout Program Quarterly Summary Report, U.S. Atomic Energy Commission A4–A288* (1970).
- Walton, A. *Rep. Isotopes Inc.* 1–37 (1960).
- Ambach, W. & Dansgaard, W. *Earth planet. Sci. Lett.* **8**, 311–316 (1970).
- Hammer, C. U. *et al. J. Glaciol.* (in the press).
- Stauffer, B. & Berner, W. *Int. Glaciol. Soc. Symp. Phys. Chem. Ice* (in the press).
- Macdonald, G. A. *Volcanoes* 1–510 (Prentice-Hall, New Jersey, 1972).
- Echevin, M., thesis, Univ. Grenoble (1971).
- Weiss, H., Bertine, K., Koide, M. & Goldberg, E. D. *Geochim. cosmochim. Acta* **39**, 1–10 (1975).
- Zoller, W. H., Gladney, E. S. & Duce, R. A. *Science* **183**, 198–200 (1974).
- Weiss, H. V., Koide, M. & Goldberg, E. D. *Science* **172**, 261–263 (1971).
- Manley, G. *Arch. Met. Wien* **9B**, 413–433 (1959).
- Schneider, Stephen, H. & Mass, Clifford *Science* **190**, 741–746 (1975).
- Baldwin, B., Pollack, J. B., Summers, A., Toon, O. B., Sagan, C. & Camp, W. *Nature* **263**, 551–555 (1976).
- Gudmandsen, P. *J. Glaciol.* **15**, 95–101 (1975).

## Nucleotide sequence and amplification in bacteria of structural gene for rat growth hormone

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*The primary structure of DNA containing the sequence for rat pituitary growth hormone mRNA has been determined. DNA was obtained by reverse transcription of polyadenylated RNA from cultured pituitary cells and from recombinant bacterial plasmids. The amino acid sequences for rat growth hormone and its precursor form have been deduced from the determined nucleotide sequences.*

SOMATOTROPIN (growth hormone, GH) is essential for linear growth in man and other vertebrates. In childhood, deficiency of this peptide hormone results in dwarfism whereas overproduction causes gigantism. In the adult, pathological overproduction of growth hormone can lead to acromegalic deformations<sup>1,2</sup>. Somatotropin is separable in activity and substance from the related pituitary hormone prolactin<sup>3,4</sup>. Comparisons of primary structures have revealed that these hormones, together with the placental hormone chorionic somatomammotropin form a group of sequence-related peptides of molecular weights (MW) around

22,000, with a suggested common evolutionary origin<sup>5-7</sup>. The three hormones also share certain features in their biological actions<sup>8</sup>.

The genes for somatotropin and related hormones thus provide an excellent system for studying structure, evolution and regulation of a set of similar DNA sequences whose expression is ordinarily confined to separate tissues (pituitary and placenta) and is elicited by different physiological stimuli. While information on the structural gene for human chorionic somatomammotropin has very recently been obtained by us<sup>9,10</sup>, no such information has been available for growth hormone.

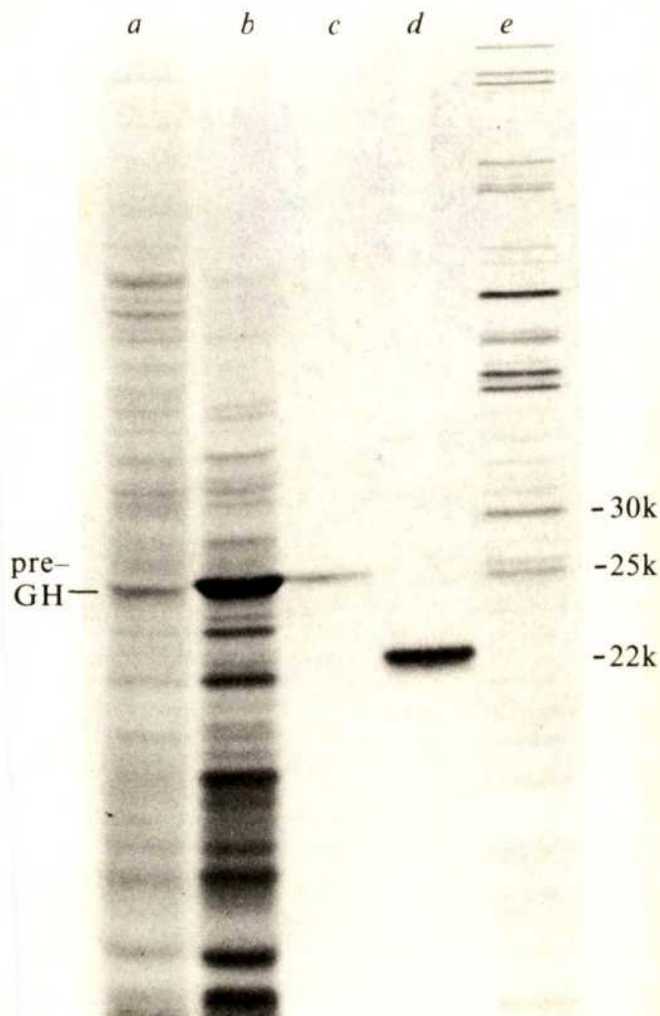
We report here the *in vitro* synthesis, determination of the nucleotide sequence, and the amplification in bacteria of structural gene sequences for growth hormone starting with mRNA from cultured rat pituitary cells<sup>11</sup>. These cells can be hormonally induced to produce and secrete somatotropin<sup>12,13</sup>. In analogy to other secreted proteins, synthesis of this peptide occurs at the rough endoplasmic reticulum as a precursor form<sup>14-16</sup>. The N-terminal portion (signal peptide) of the precursor is thought to be involved in the attachment of ribosomes to membranes, a process essential for hormone maturation and secretion (for model on mechanism see ref. 17). Accordingly, the primary translation product of rat growth hormone mRNA is a peptide larger (by about 3,000 MW units, see Fig. 1) than the mature hormone<sup>13,16</sup>. As the primary structure of rat somatotropin is only partially known<sup>18</sup>, the nucleotide sequence determined for the mRNA allows us to predict the complete amino acid sequence of the hormone including the previously unknown sequence of the signal peptide.

### Detection and sequence determination of DNA complementary to growth hormone mRNA

Certain tissues in which the mRNA for a specialised function represents a large proportion of the polyadenylated RNA (for example, globin RNA in reticulocytes<sup>19</sup>, ovalbumin RNA in induced oviducts<sup>20</sup>, insulin RNA in the islets of Langerhans<sup>21,22</sup>) allow for their convenient detection and characterisation of such RNAs or their cDNAs. In cultured rat pituitary cells (strain GC), however, when grown in normal conditions, growth hormone mRNA represents only a small percentage (1-3%) of the total poly A containing RNA<sup>13</sup>. Therefore, the detection and isolation of nucleic acid sequences specific for rat growth hormone were approached by a strategy previously used to obtain sequences from polyadenylated RNAs that constitute 2% or more of the cellular messengers<sup>9</sup>. This strategy involves restriction endonucleolytic cleavage of complex cDNA mixtures (single or double stranded) prepared by reverse transcription of polyadenylated cellular RNAs, followed by gel electrophoretic separation of the resultant cDNA fragments. In applying this approach to the detection of growth hormone cDNA we were aided by the fact that in cultured rat pituitary cells growth hormone mRNA levels can be raised above that of other cellular mRNA species by the synergistic action of thyroid hormones and glucocorticoids<sup>13,23</sup>. Further enrichment of growth hormone mRNA was achieved by isolating poly A-containing RNA from cytoplasmic membranes<sup>24</sup> where growth hormone is synthesised. In the final RNA template used for cDNA synthesis, growth hormone mRNA was therefore the most abundant species representing about 10% of the RNA (see Fig. 1).

Analysis by gel electrophoresis of this cDNA after cleavage with endonucleases *Hae*III and *Hha*I is shown in Fig. 2. Each enzyme generates several abundant DNA fragments which can be seen as prominent bands above the polydisperse background of the restricted cDNA. Whereas *Hae*III yields a complex fragment pattern (Fig. 2b), cleavage with *Hha*I produces a simpler pattern characterised by two major DNA fragments, approximately 320 (fragment A) and 240 (fragment B) nucleotides long (Fig. 2c). The prominence of fragments A and B suggests that they may be derived from growth hormone mRNA.

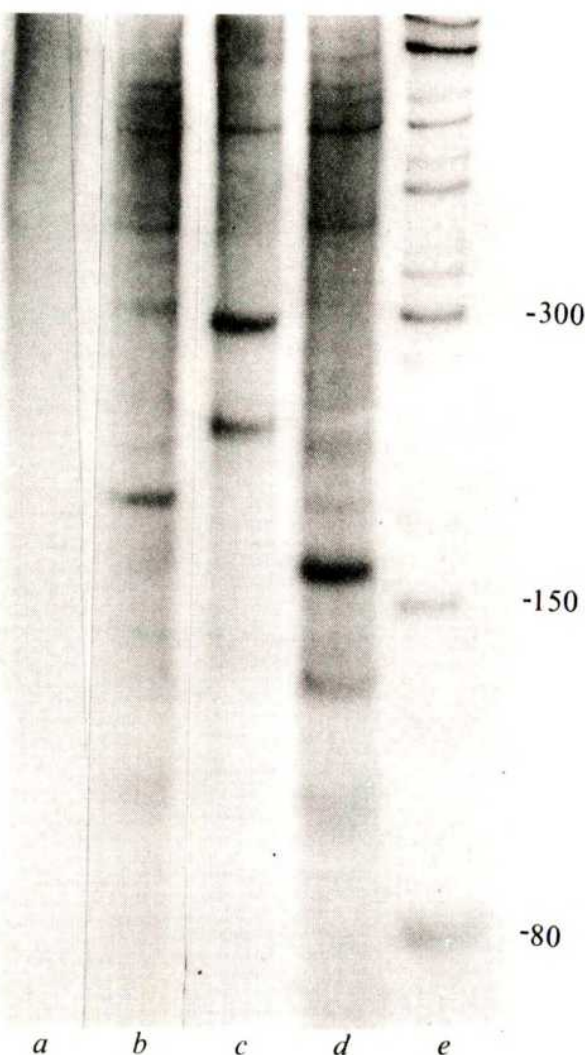
For further characterisation, these fragments were isolated from a polyacrylamide gel on which approximately 1  $\mu$ g of *Hha*I-



**Fig. 1** Translation products of rat growth hormone mRNA. Polyadenylated RNA was isolated from the cytoplasmic membrane fraction of cultured rat pituitary cells (strain GC) as described elsewhere<sup>13,24</sup>. Usually, 30  $\mu$ g of poly A-containing RNA were obtained from  $5 \times 10^8$  cells grown in suspension culture and induced for GH production by including 1  $\mu$ M dexamethasone and 10 nM L-triiodothyronine in the medium for 4 d before cell collection. Polyadenylated RNA was also isolated from cells grown for 4 d in the absence of dexamethasone and triiodothyronine<sup>23</sup>. Aliquots of these RNA preparations (500 ng) were translated in a cell-free translation system derived from wheat germ, and the products immune-precipitated using rhesus monkey antiserum to rat GH as previously described<sup>13</sup>. To obtain labelled rat growth hormone, induced cells (about  $10^6$  cells in 1 ml) were pelleted, resuspended in 0.2 ml of serum- and methionine-free medium containing bovine serum albumin (0.5  $\mu$ g ml<sup>-1</sup>), and labelling was carried out with <sup>35</sup>S-methionine (50  $\mu$ Ci, NEN) for 4 h at 37 °C. Cells were pelleted and labelled rat GH released into the medium was immune-precipitated as described above. Protein products of cell-free translations and immunoprecipitations were separated in 12.5% polyacrylamide slab gels (14  $\times$  14  $\times$  0.2 cm) in the presence of sodium dodecylsulphate<sup>26</sup> at 20 mA per gel for 4 h. Gels were soaked in 50% trichloroacetic acid, washed in 7% acetic acid, dried on paper (Whatman 3MM), and exposed to X-ray film (Kodak NS2T). The autoradiogram shown contains a, translation products of poly A 'membrane' RNA from cells grown in the absence of dexamethasone and thyroid hormone; b, the same as (a) except that the RNA was isolated from hormone-induced cells; c, immune-precipitate of (b); d, immune-precipitate of secreted rat GH; e, <sup>14</sup>C-labelled proteins from bacteriophage T4-infected *E. coli* cells<sup>27</sup> as size markers.

restricted cDNA had been separated. Each fragment was further cleaved by *Hae*III (see Fig. 2c) and the products (A<sub>1</sub> ~ 180, A<sub>2</sub> ~ 75, A<sub>3</sub> ~ 65, B<sub>1</sub> ~ 140, B<sub>2</sub> ~ 100 nucleotides) enzymatically labelled with <sup>32</sup>P at their 5' termini. Following gel electrophoretic separation, the five labelled DNA fragments were processed for nucleotide sequence determination according to





**Fig. 2** Restriction endonuclease cleavage of single-stranded cDNA to rat pituitary cell RNA. Aliquots (300 ng) of the polyadenylated RNA prepared as described in Fig. 1, were transcribed into cDNA in 5- $\mu$ l reactions containing 50 ng oligo-dT<sub>12-18</sub> (Collaborative Research), 50 mM Tris-HCl, pH 8.1, 20 mM KCl, 7 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 10 mM  $\beta$ -mercaptoethanol, 500  $\mu$ M each of dGTP, dCTP, dTTP, 30  $\mu$ M  $\alpha$ -<sup>32</sup>PdATP (specific activity  $5 \times 10^4$  c.p.m. pmol<sup>-1</sup>), and 5 U reverse transcriptase. DNA synthesis proceeded for 7 min at 42 °C and incorporation of <sup>32</sup>P-dAMP into DNA was approximately  $2 \times 10^6$  c.p.m. Reaction mixtures were diluted with 20  $\mu$ l of H<sub>2</sub>O, heated to 95 °C for 2 min, quick-cooled, adjusted to 7 mM MgCl<sub>2</sub>, and split into 5  $\mu$ l aliquots for restriction endonuclease cleavage of cDNA. Digestion with *Hae*III (2 U), or *Hha*I (2 U), or both enzymes<sup>9</sup> was for 60 min at 37 °C. Mixtures were made 10 mM in EDTA, 10% in sucrose, 0.2% in sarcosyl, 0.05% in bromophenol blue (final volume 10  $\mu$ l), heated to 95 °C for 2 min, quick-cooled, loaded on to 4.5% polyacrylamide slab gels (14  $\times$  14  $\times$  0.2 cm) in TBE buffer<sup>58</sup>, and electrophoresed at 150 V for 2 h. Gels were dried and exposed to X-ray film (Kodak NS2T). The autoradiogram shown (obtained after 4 h of exposure) contains a, uncleaved cDNA; b, *Hae*III-cleaved cDNA; c, *Hha*I-cleaved cDNA; d, cDNA cleaved with *Hae*III and *Hha*I; e, *Hae*III-cleaved <sup>32</sup>P-labelled phage M13 single-stranded DNA<sup>59</sup> as molecular weight marker. Numbers indicate approximate sizes in nucleotides.

Maxam and Gilbert<sup>25</sup>. Typical examples of such determinations are shown in Fig. 3, and the nucleotide sequences are compiled in Fig. 7.

To identify their origin, the cDNA sequences were converted into their antiparallel complementary RNA sequences and the coding potential in the three possible phases was compared with the known amino acid sequences of several vertebrate GHs<sup>26</sup>, including parts of rat growth hormone determined by Wallis and Davies<sup>18</sup>. This comparison showed that cDNA fragments A and B clearly originate from rat growth hormone mRNA. This result emphasises the experimental advantage of analysing DNA reverse transcribed from complex cellular RNA mixtures for

obtaining primary structural information on abundant mRNA species.

Fragments A and B are complementary to a contiguous segment of the mRNA, extending from the codon for amino acid 149 to approximately 40 nucleotides to the 5' side of the AUG initiation codon for pre-growth hormone (see Fig. 8). Since the distance of some 40 nucleotides from the end of fragment B to the initiation codon for pre-growth hormone (see Fig. 8). As the region in other eukaryotic mRNAs<sup>27,28</sup>, it is possible that the 3' terminus of fragment B corresponds to the 5' terminus of the mRNA, and is not generated by *Hha*I cleavage (see also later).

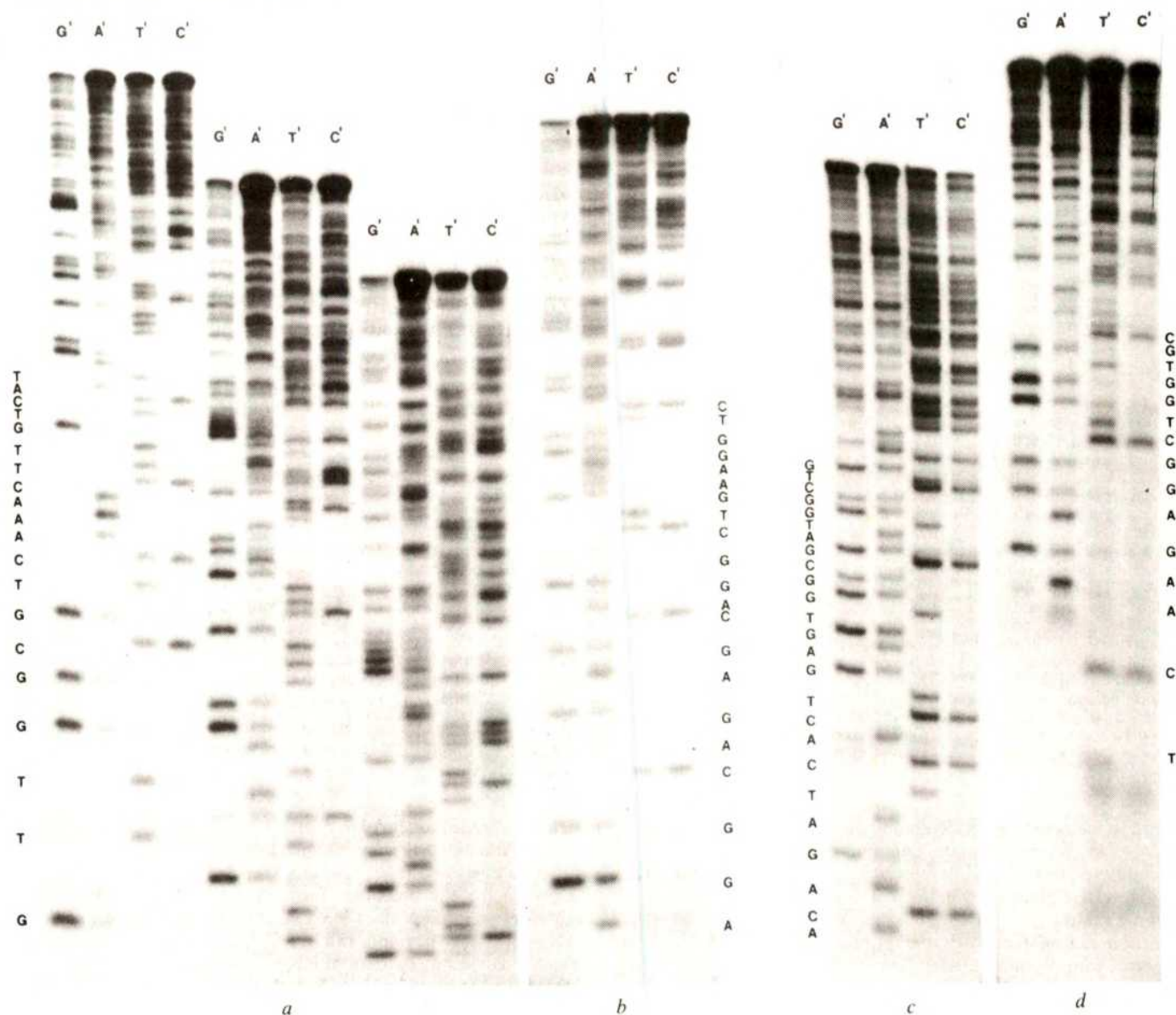
### Molecular cloning of growth hormone structural gene sequences

To obtain a convenient source of growth hormone DNA for further analyses, we constructed bacterial plasmids containing the structural gene for rat growth hormone. The cDNA fragments A and B served as excellent markers for monitoring construction of the correct plasmids. The starting material for the *in vitro* synthesis and subsequent cloning of the structural gene sequences was a cDNA preparation comparable in amount and purity to the one used for sequencing fragments A and B. By using its self-priming properties, this cDNA was enzymatically converted to its duplex form characterised by a hair-pin structure on one end and a poly(dA-dT) tract on the other<sup>29</sup>. S1 nuclease was used to open the terminal hair-pin loop and to degrade any single-stranded DNA. Gel analysis of the DNA material before and after nuclease treatment (Fig. 4b, c) demonstrates the presence of a double-stranded DNA of approximately 800 base pairs faintly detectable above the disperse background of other DNA. An aliquot of this DNA species was purified by gel electrophoresis and cleaved with *Hha*I. Two prominent fragments corresponding in size to DNA fragments A and B were among the major cleavage products (see Fig. 4i). As fragment B is complementary to the 5' portion of growth hormone mRNA (see above), this finding indicated that the 800-base pair DNA contained primarily full-length copies of the messenger.

To allow for reversible insertion into a bacterial cloning vehicle, the double-stranded cDNA was given cohesive ends by enzymatic blunt-end ligation<sup>30</sup> to chemically-synthesised restriction site linkers, and subsequent cleavage with the appropriate restriction endonuclease<sup>31,32</sup>. The linkers had the self-complementary sequence 5'-CCAAGCTTGG-3', containing the recognition site for *Hind*III. This site is not present in the 800-base pair DNA (data not shown) and occurs once in the bacterial plasmid pBR322 chosen for cDNA insertion (see below). Preceding ligation to linkers, the S1 nuclease-treated double-stranded cDNAs were 'blunt-ended' using *Escherichia coli* DNA polymerase I in the presence of deoxynucleoside triphosphates<sup>33</sup> (see legend to Fig. 4). This step ensured a high participation of the cDNA molecules in the ligation reaction, as shown by the blurred appearance due to multiple linker additions, of both the 800-base pair DNA and DNA fragments A and B (see Fig. 4d, f). Fragments A and B had been obtained by *Hha*I-cleavage of the double-stranded cDNA before S1-treatment and their 3'-CG ends removed by *E. coli* DNA polymerase I before linker attachment. The ligation products were cleaved with *Hind*III (*Hsu*I endonuclease was used interchangeably with *Hind*III) to generate DNA molecules with cohesive termini and to digest self-ligated linkers (Fig. 4e, g). The 800-base pair DNA and DNA fragments A and B were purified on a polyacrylamide gel (Fig. 4h, k) to reduce the level of DNA sequences unrelated to growth hormone that would enter the bacterial cells.

The EK-2 vector pBR 322<sup>34</sup>, a relaxed replicating plasmid of MW  $2.6 \times 10^6$  containing a single *Hind*III cleavage site was used as the cloning vehicle for growth hormone cDNA. Before insertion of the cDNA, the *Hind*III-cleaved linear plasmid was treated with bacterial alkaline phosphatase to remove the 5'-terminal phosphate groups. This step permits the selective construction of recombinant plasmids by preventing closure of the plasmid at the original cleavage site. Infectious circular plasmid forms will only be generated by bridging and closing the plasmid

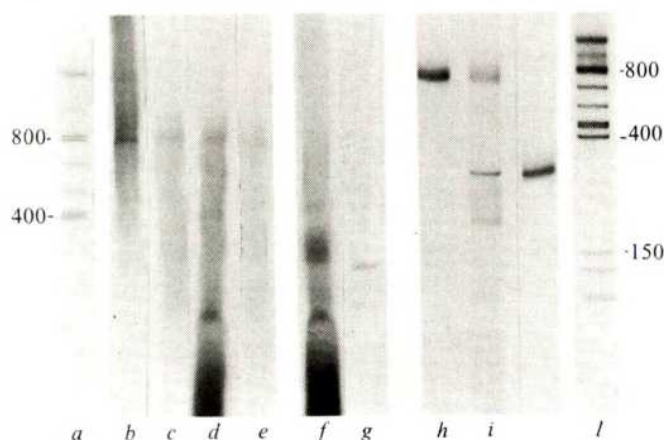




**Fig. 3.** Autoradiograms of 20% polyacrylamide, 7 M urea gels used for sequence analysis of *a*, cDNA fragment A<sub>1</sub> (nucleotide 522 to 340, see Fig. 7), starting at nucleotide 519; *b*, cDNA fragment B<sub>2</sub> (60 to about -40), starting at nucleotide 53; *c*, 5' terminal *AluI* fragment (-22 to 144) of pRGH-1, starting at nucleotide -18; *d*, *HaeIII* fragment (61 to 274) of pRGH-1, starting at nucleotide 63. The labelled DNA fragments were recovered from polyacrylamide slab gels (see below) and subjected to the described procedure for nucleotide sequence analysis<sup>2,5</sup>. G, A, T, C refer to the respective base specific cleavages. The nucleotide sequence can be read directly from the gel as shown for the 5' portion of each sequence fragment. Single-stranded cDNA for nucleotide sequence analysis was prepared from polyadenylated RNA as described in the legend to Fig. 2, except that the reaction (200 µl) contained 15 µg of RNA, 2 µg of oligo dT<sub>12-18</sub>, 800 µM each of dGTP, dCTP, dTTP, and 400 µM of α-<sup>32</sup>P-dATP (specific activity 500 c.p.m. pmol<sup>-1</sup>). Synthesis proceeded for 10 min at 42 °C (incorporation of <sup>32</sup>P-dAMP into cDNA was about 5 × 10<sup>5</sup> c.p.m.), and was stopped by addition of EDTA to 10 mM. Reaction mixtures were phenolised and the nucleic acid precipitated with ethanol. Pellets were then dissolved in 100 µl of Tris-HCl, pH 7.5, 0.01 mM EDTA, 40 mM NaCl, 5 mM β-mercaptoethanol, heated to 95 °C for 5 min, quick-cooled, and adjusted to 7 mM MgCl<sub>2</sub>. After addition of *HhaI* (20 U) digestion proceeded for 2 h at 37 °C, followed by incubation with 0.2 U of bacterial alkaline phosphatase (BAPE, Worthington) for 10 min at 65 °C. Reaction mixtures were phenolised, DNA precipitated with ethanol, redissolved in 25 µl of 5 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% sucrose, 0.05% bromophenol blue, and separated on 6% polyacrylamide slab gels in TBE buffer<sup>58</sup> for 2 h at 150 V. cDNA fragments were visualised by exposing the wet gels to X-ray film (Kodak, NS2T) for 3 h. Gel bands containing cDNA fragments A and B were excised, DNA was eluted overnight from the crushed gel material<sup>2,5</sup> and precipitated with ethanol. The recovered fragments were cleaved with *HaeIII* (5 U per fragment) at 37 °C for 2 h in 20 µl of 10 mM Tris-HCl, pH 7.5, 8 mM MgCl<sub>2</sub>. After inactivation of *HaeIII* at 80 °C for 5 min, the DNA fragments were terminally labelled by adjusting the digestion mixtures to 50 mM Tris-HCl, pH 8.5, 10 mM MgCl<sub>2</sub>, 0.1 mM spermidine, 5 mM β-mercaptoethanol, 5% glycerol, 500 µCi γ-<sup>32</sup>P-ATP (specific activity 1.5 µCi pmol<sup>-1</sup>), and 5 U of T4 polynucleotide kinase at 37 °C for 2 h (final volume 40 µl). Following phenol extraction, the DNA was precipitated with ethanol, dissolved in 25 µl of mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% sucrose, 0.05% bromophenol blue, and separated by electrophoresis in 8% polyacrylamide slab gels. cDNA fragments were visualised by autoradiography of the wet gels (exposure time about 10–20 min), excised and eluted from the gels, precipitated with ethanol, and processed directly for nucleotide sequence determination<sup>2,5</sup>. For nucleotide sequence analysis of pRGH-1 DNA an appropriate amount (5–30 µg) of the isolated 800-base pair DNA insert was cleaved with one, or a combination, of the restriction endonucleases *HaeIII*, *HhaI*, *AluI*, *HpaII*, *KpnI* or *PstI* (see Fig. 6). After 5'-terminal enzymatic labelling (see above) fragments (isolated individually or in a mixture) were additionally cleaved with one of the enzymes listed above, and products were separated by polyacrylamide slab gel electrophoresis. In cases where a DNA fragment labelled at both 5' termini could not be cleaved by one of the enzymes listed above, the two strands of that fragment were separated on a 8% polyacrylamide slab gel in TBE buffer, after loading in 0.2 M NaOH, 1 mM EDTA<sup>2,5</sup>.

Following ligation of the gel-isolated 800-base pair DNA to phosphatase-treated linear pBR322 plasmid the reaction mixture





(see above). Fractions containing DNA were pooled and the nucleic acid precipitated with ethanol. The precipitate was redissolved in 100  $\mu$ l of 5 mM Tris-HCl, pH 7.5, 0.05 mM EDTA, and split into 50  $\mu$ l aliquots. One aliquot was adjusted to 300 mM NaCl, 30 mM Na-acetate, pH 4.5, 3 mM ZnCl<sub>2</sub>, and incubated with 5 U of S1 nuclease (prepared according to Vogt<sup>60</sup>) at 37 °C for 10 min followed by phenol extraction in the presence of 5 mM EDTA and precipitation of the DNA with ethanol. The second aliquot was adjusted to 10 mM Tris-HCl, pH 7.5, 30 mM NaCl, 8 mM MgCl<sub>2</sub>, 5 mM  $\beta$ -mercaptoethanol, and the DNA was cleaved with 5 U of *Hha*I for 2 h at 37 °C followed by phenol extraction and precipitation of the DNA fragments with ethanol. For ligation to the *Hind*III linkers, DNA precipitates from both aliquots were separately dissolved in 25  $\mu$ l of 60 mM Tris-HCl, pH 7.5, 8 mM MgCl<sub>2</sub>, 10 mM  $\beta$ -mercaptoethanol, 1 mM ATP, and 200  $\mu$ M each of dATP, dTTP, dGTP, dCTP. They were incubated with 1 U (1  $\mu$ l) of *E. coli* DNA polymerase I at 10 °C for 10 min to exonucleolytically remove any 3' protruding ends and to 'fill in' any 5' protruding ends<sup>33</sup>. Incubation was followed by addition of 50 pmol of 5' <sup>32</sup>P-labelled *Hind*III linker (10<sup>5</sup> c.p.m. pmol<sup>-1</sup>, in 15  $\mu$ l of 60 mM Tris-HCl, pH 7.5, 8 mM MgCl<sub>2</sub>, 10 mM  $\beta$ -mercaptoethanol, 1 mM ATP), and bacteriophage T4 DNA ligase (to 20  $\mu$ g ml<sup>-1</sup>)<sup>61</sup>. Ligation was for 60 min at 20 °C. Reaction mixtures were diluted with 100  $\mu$ l of 7 mM MgCl<sub>2</sub>, 50 mM NaCl, heated at 80 °C for 5 min, cooled and DNA digested with 15 U of *Hsu*I at 37 °C for 4 h. The reaction mixtures were phenolised, DNA was precipitated with ethanol, redissolved in 25  $\mu$ l of 5 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% sucrose, 0.05% bromophenol blue, and separated on 4.5% polyacrylamide slab gel at 150 V for 2 h. The 800-base pair DNA and the double-stranded rat GH DNA fragments A and B were visualised by autoradiography of the wet gel and electrophoretically eluted from the isolated gel pieces. Lanes a and l, *Hpa*II-cleaved <sup>32</sup>P-labelled phage M13 RF DNA<sup>62</sup> as molecular weight marker (numbers indicate approximate sizes in base pairs); b, DNA product after second strand synthesis; c, S1 nuclease digest of the sample shown in b; d, *E. coli* DNA polymerase I treatment and ligation of *Hind*III linkers to the sample shown in c; e, digestion of sample d with *Hsu*I; f, double-stranded cDNA cleaved with *Hha*I, blunt-ended with *E. coli* DNA polymerase I, and ligated to *Hind*III linkers; g, *Hsu*I cleavage of the sample shown in f; h 800-base pair DNA with cohesive *Hind*III termini after preparative gel elution; i, *Hha*I digestion of the gel-eluted 800-base pair DNA; k, double-stranded DNA fragment A with cohesive *Hind*III termini after preparative gel elution.

tetracycline resistance<sup>34</sup>. Recombinants were therefore selected by growth on nutrient plates containing ampicillin, and by their inability to grow on high levels (20  $\mu$ g ml<sup>-1</sup>) of tetracycline. Ten colonies were obtained which all carried plasmid with an insert of approximately 800 base pairs that was released by *Hind*III cleavage. Consistent with the purity of the cDNA material inserted into plasmid (30–50% growth hormone DNA, see Fig. 4i), 4 of the 10 inserts were growth hormone DNA as judged by their restriction endonuclease patterns (Figs 5 and 6), and shown by direct sequencing in one case (see below).

Growth hormone DNA fragment A was successfully cloned in the same manner: 7 out of 8 clones contained plasmid with the correct DNA insertion as seen by *Hae*III and *Kpn*I digestion (see Fig. 6). Nucleotide sequence analysis of one of these inserts showed that the 3' protruding –CG termini in the double-stranded cDNA fragment A had been removed correctly by *E. coli* DNA polymerase I (see Fig. 7) demonstrating the reliability of this method for obtaining defined blunt-ended DNA molecules from those with frayed ends. No clones were obtained for DNA fragment B. This could reflect the finding (see below) that the cloned 800-base pair DNA does not contain the presumptive *Hha*I-site which was thought to terminate the single-stranded cDNA fragment B. The double-stranded form of this fragment probably retained its terminal hairpin after *Hha*I cleavage, thereby preventing the addition of linkers to this end of the molecule and its subsequent insertion into the cloning vehicle.

### Sequence determination of the cloned DNA

The 800-base pair growth hormone DNA was isolated in preparative amounts from one of the recombinant clones (pRGH-1) and its nucleotide sequence determined by the method of Maxam and Gilbert<sup>25</sup> (see Fig. 3b). The entire sequence of this DNA is compiled in Fig. 7. A large part of it has been confirmed by determining the nucleotide order in both DNA strands. The sequence is identical to that determined for cDNA fragments A and B which demonstrates the fidelity of amplifying eukaryotic

Fig. 4 Preparation of double-stranded cDNA from rat growth hormone mRNA. Polyadenylated pituitary cell RNA (15  $\mu$ g) was reverse-transcribed into cDNA as detailed in Fig. 3. Synthesis was stopped by addition of EDTA to 10 mM, the reaction mixture phenolised and nucleic acid precipitated with ethanol. The precipitate was redissolved in 75  $\mu$ l of 5 mM Tris-HCl, pH 7.5, 1 mM EDTA, fractionated on a Sephadex G-50 column (0.6  $\times$  10 cm) in the same buffer, and fractions containing the cDNA were pooled. After precipitation with ethanol nucleic acid was redissolved in 150  $\mu$ l of 0.1 N NaOH, 1 mM EDTA, and the RNA hydrolysed at 70 °C for 20 min. After neutralisation and precipitation with ethanol, the single-stranded cDNA was converted to its duplex form in a 50- $\mu$ l reaction containing 50 mM Tris-HCl, pH 8.1, 0.1 mM EDTA, 20 mM KCl, 7 mM MgCl<sub>2</sub>, 10 mM  $\beta$ -mercaptoethanol, 400  $\mu$ M each of dGTP, dTTP, dCTP, and 100  $\mu$ M of  $\alpha$ -<sup>32</sup>P dATP (specific activity about 10<sup>4</sup> c.p.m. pmol<sup>-1</sup>). The reaction mixture was heated to 90 °C for 2 min, quick-cooled, and synthesis proceeded for 2 h at 40 °C (incorporation of <sup>32</sup>P-dAMP into DNA was approximately 3  $\times$  10<sup>6</sup> c.p.m.) followed by addition of 2  $\mu$ g of *E. coli* tRNA, phenol extraction, and fractionation of the aqueous phase by gel filtration

structural gene sequences in bacteria, and stresses the advantage of cDNA sequencing to probe such fidelity.

The cloned 800-base pair DNA specifies the entire coding sequence for the precursor form of rat growth hormone. In addition, it contains all of the 3', and part of the 5' untranslated region of growth hormone mRNA. Failure to obtain the complete 5'-terminal sequence of a mRNA in cloned double-stranded cDNA (synthesised *in vitro* by self-priming of single-stranded cDNA and S1 nuclease treatment) has also been observed in the few other instances reported<sup>22,35</sup>.

### Primary structure of rat growth hormone mRNA

The DNA sequence determined from cDNA and cloned material allows us to derive the primary structure of rat growth hormone mRNA as shown in Fig. 8. The size of this RNA (excluding the poly A) is estimated to be 800 nucleotides, assuming that the 5' end corresponds approximately to the 3' end of cDNA fragment B. This estimate agrees well with previous values obtained from velocity sedimentation<sup>13</sup> and gel electrophoresis<sup>36</sup> of rat growth hormone mRNA.

Only preliminary conclusions can be drawn about the 5'-untranslated part of growth hormone RNA since the full sequence has not yet been established. At least 20 nucleotides are known to be missing from pRGH-1, but a tentative sequence is available from cDNA fragment B (Fig. 7).

No obvious features characterise the established 5' portion of the mRNA sequence and no apparent homology to known 5' untranslated regions of other eukaryotic messengers is found. But, there is a potential complementarity with the 3' terminal sequence (5'-GAUCAUUA-3') of eukaryotic 18S ribosomal RNA involving nucleotides –15 to –12 in the messenger. An interaction between the 5' end of mRNA and the 3' end of ribosomal RNA has been demonstrated in the initiation of translation for prokaryotic mRNAs<sup>37,38</sup>, and by analogy has been suggested to occur in eukaryotes<sup>39</sup>. The linear distance from this site to the initiation codon AUG is comparable with that in rabbit  $\beta$ -globin RNA<sup>27,40</sup> and rous sarcoma viral RNA<sup>28</sup> where



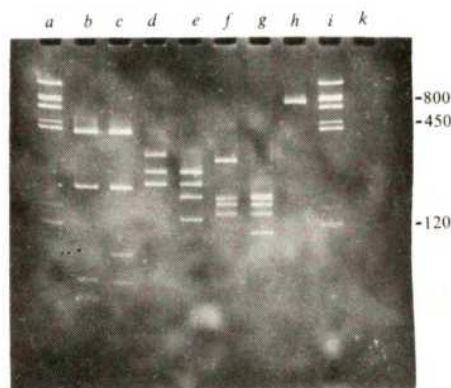
such a complementarity has also been observed.

It may be of interest that the region surrounding the AUG initiation codon can be folded into a secondary structure with the AUG being part of a hairpin loop (residues -4 to 3) and the base-paired stem interrupted by non-complementary sequences (residues -17 to -11, and 11 to 20) containing the region complementary to the 3' terminus of 18S ribosomal RNA. Such a structure ( $T_m$  approximately 50 °C) could form through interaction with proteins involved in the initiation of protein synthesis.

The 3' untranslated region of rat GH mRNA contains 105 nucleotides, excluding the poly A. Whereas the distal portion of this region is rich (73%) in A+U, 30 of the first 64 nucleotides following the UAG termination codon are C's, present mainly in stretches of 4, 5, or 6 residues. Two of these C tracts are part of a sequence of 16 pyrimidines (residues 676-693) interrupted around the centre by two purines. Such highly asymmetric distribution of purines and pyrimidines is known to influence the secondary structure of the corresponding DNA double helix<sup>42</sup> and may be of significance concerning structural requirements for transcriptional regulation. It is interesting to note that the CCACCCU sequence (nucleotides 709-716) found at the end of the C-rich region is also present in rat insulin mRNA<sup>22</sup>, and slightly modified in human  $\alpha$ -globin<sup>43</sup> and rabbit  $\beta$ -globin RNA<sup>44</sup>.

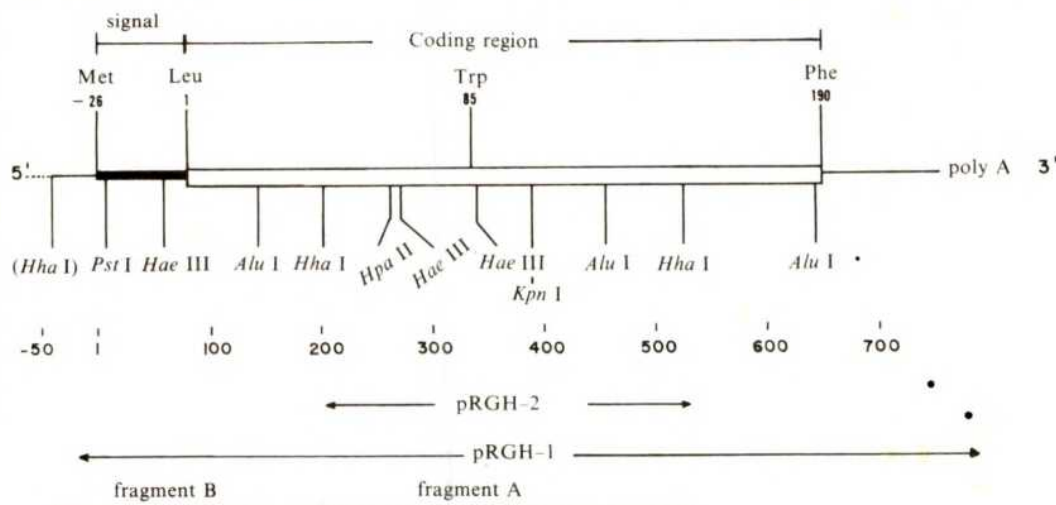
Several regions with symmetry elements occur throughout the 3'-terminal sequence of GH mRNA. Of note are four regions of a palindromic nature the first two of which (following the termination codon) overlap in sequence comprising 12 and 20 nucleotides and are centred around positions 656 and 667 respectively. The third region surrounds the GUUA sequence (residues 683-686) with two C tracts, and is reminiscent of the extended palindrome found in an equivalent position in HCS mRNA<sup>9,10</sup>. The fourth palindrome (nucleotides 728-740) is solely composed of A and U residues and contains the AAUAAA stretch found in all other eukaryotic mRNAs sequenced to date<sup>45</sup>. Between this sequence and the poly A tract a self-complementary sequence occurs (nucleotides 739-750) which would form a region of twofold symmetry in the corresponding double-stranded DNA. The functions of these symmetry elements and sequence features are unknown. These sequences may be of regulatory nature providing sites of interaction with proteins involved in the transcription of the growth hormone gene, and in the processing, packaging, and translation of growth hormone mRNA.

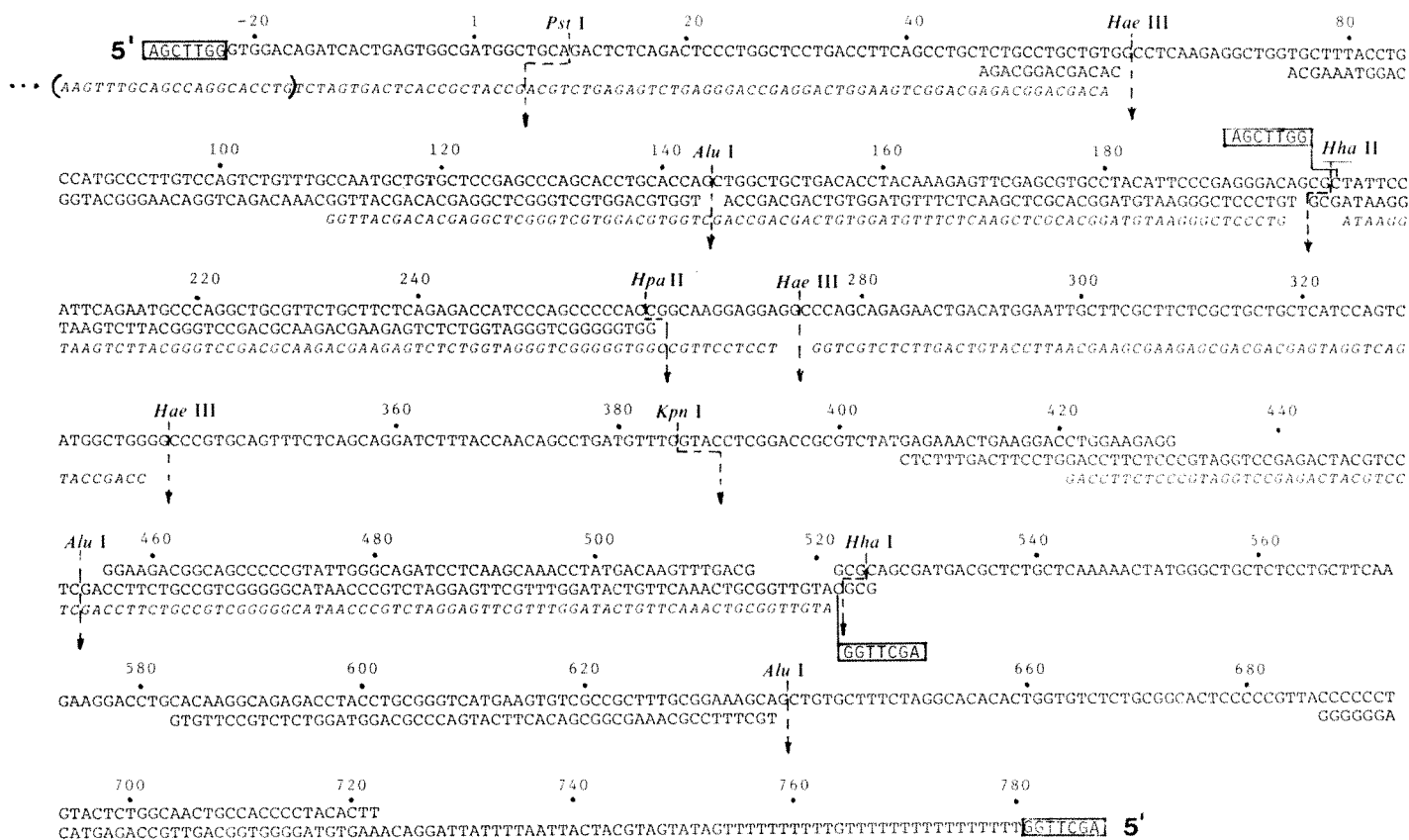
The poly A tract characteristic of most eukaryotic mRNAs<sup>46</sup> starts with nucleotide 754. As determined from the cloned DNA sequence this tract is interrupted after 10 nucleotides by a C residue. This finding probably reflects the slippage of the extended oligo-dT primer in the initial stages of cDNA synthesis. But, the existence of occasional substitutions in poly A tracts of mRNAs cannot be excluded.



**Fig. 5** Restriction endonuclease cleavage pattern of cloned 800-base pair rat growth hormone DNA. The 800-base pair DNA and DNA fragments A and B, purified by gel electrophoresis, were ligated to the EK-2 vector pBR 322<sup>34</sup> in 50- $\mu$ l reactions containing 60 mM Tris-HCl, pH 8, 10 mM  $\beta$ -mercaptoethanol, 8 mM MgCl<sub>2</sub>, between 10 and 50 ng (as judged from their specific activities) of the purified DNAs, and approximately 500 ng of *Hind*III-cleaved 5'-dephosphorylated plasmid DNA. Ligations were started by addition of T4 DNA ligase (to 5  $\mu$ g ml<sup>-1</sup>)<sup>61</sup>, allowed to proceed at 15 °C for 1 h, and mixtures diluted to 0.25 ml with 120 mM NaCl, 1 mM EDTA. The NIH-certified EK-2 host *E. coli* X1776 constructed by R. Curtiss III was grown in 150 ml of nutrient broth supplemented with DL- $\alpha$ -diaminopimelic acid (DAP, 100  $\mu$ g ml<sup>-1</sup>) and thymine (40  $\mu$ g ml<sup>-1</sup>) to an  $A_{650}$  of 0.2. After centrifugation the cells were washed in 60 ml of 10 mM NaCl, pelleted, resuspended in 60 ml of transformation buffer (10 mM Tris-HCl, pH 8, 140 mM NaCl, 75 mM CaCl<sub>2</sub>), kept on ice for 15 min, pelleted, and resuspended in 1.5 ml of the same buffer. The following transformation, cell growth, and the isolation of cloned, recombinant plasmid DNA were performed in a certified P3 laboratory. After adding 0.5 ml of CaCl<sub>2</sub>-treated cells to the diluted ligation reactions the transformation mixtures (0.75 ml) were kept on ice for 15 min, at 25 °C for 4 min, and again on ice for 30 min. They were then plated directly on to nutrient plates (about 0.2 ml per plate) containing ampicillin (20  $\mu$ g ml<sup>-1</sup>), DAP (100  $\mu$ g ml<sup>-1</sup>), and thymine (40  $\mu$ g ml<sup>-1</sup>) and the plates incubated for 24 h at 37 °C. In these conditions about 20 transformants were usually obtained from each ng of supercoiled pBR 322 plasmid. In the experiments described here transformation with the 800-base pair DNA yielded 11 colonies, one of which grew on tetracycline (20  $\mu$ g ml<sup>-1</sup>) and carried plasmid without insert. cDNA fragment preparations A and B gave 8 and 5 colonies, respectively. Analysis of the isolated plasmids by restriction endonuclease cleavage and 6% polyacrylamide slab gel electrophoresis of the cleavage products (which were then visualised under ultraviolet light after staining with ethidium bromide<sup>66</sup>) showed that 4 colonies contained 800-base pair GH DNA, 7 colonies contained GH DNA fragment A and none was found to contain GH fragment B. Shown are digests of pRGH-1 insert (800-base pair rat GH DNA) with the restriction endonucleases: *Hae*III and *Pst*I, (b); *Hae*III, (c); *Hha*I, (d); *Hha*I and *Kpn*I (e); *Alu*I (f); *Alu*I and *Hpa*II (g); no enzyme (h). Lanes (a) and (i) contain *Hpa*II-cleaved phage fd-RF DNA<sup>62</sup> as molecular weight marker. Numbers indicate approximate sizes in base pairs.

**Fig. 6** Physical map of rat growth hormone mRNA from the poly A to the 5' end. The black and white boxes span the coding region and represent the signal region and the region coding for the mature hormone respectively. Characteristic amino acids and their respective positions are shown above the boxed areas for orientation. The locations of the cleavage sites in the corresponding DNA for restriction endonucleases *Alu*I, *Hae*III, *Hha*I, *Hpa*II, *Kpn*I, and *Pst*I were determined from growth hormone cDNA and from cloned growth hormone DNA. It is uncertain whether the left-most *Hha*I site exists or whether this site represents the 5' end of growth hormone mRNA. The segments comprised by cDNA fragments A and B, and by the cloned fragments present in pRGH-1 and pRGH-2 are indicated. Nucleotide numbers are given below the symbols for the endonucleases.





**Fig. 7** Nucleotide sequence of the structural gene for rat growth hormone. The sequences shown were determined by the method of Maxam and Gilbert<sup>25</sup> from fragments of single-stranded cDNA to rat growth hormone mRNA and from the recombinant bacterial plasmids pRGH-1 and pRGH-2 (see Fig. 3). Also shown are the locations of restriction endonuclease sites used for cleaving cDNA and cloned DNAs. The sequences obtained from the single-stranded cDNA fragments, A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, B<sub>1</sub>, and B<sub>2</sub> are in italics and span residues 521–421, 272–202, 337–276, 196–110, and 58 to –37 respectively. (Position 1 is assigned to the A residue in the AUG initiator for the precursor protein.) The cDNA sequence in parentheses is not fully confirmed. Sequences obtained from cloned DNAs are shown in bold type. The second C residue in the sequence CC<sup>1</sup>AGG appears as a gap in the sequencing gels due to methylation<sup>63</sup> in *E. coli* X1776. The presence of this nucleotide inferred from band spacings was confirmed from the sequence of the complementary strand or the cDNA. Nucleotides 523–525 were not directly sequenced but inferred from the existence of the *Hha*I site in this position. The boxed sequences represent the cohesive termini derived from the *Hind*III linkers and mark the ends of pRGH-1 and pRGH-2.

The coding region contains 648 nucleotides which specify the 216 amino acids of the growth hormone precursor. The codon choices are listed in Table 1. It can be seen that codon utilisation is distinctly non-random, a phenomenon also noted in the coding sequences of other eukaryotic<sup>9,10,22,40</sup> and prokaryotic<sup>47,48</sup> mRNAs. As a consequence of such selective codon usage, growth hormone mRNA has a high G + C content (57.7%) which exceeds that of total rat DNA (43%)<sup>49</sup>.

### Amino acid sequence of rat pre-growth hormone

The established mRNA sequence permits the prediction of the complete amino acid sequence of the proposed physiological precursor form of rat growth hormone (pre-GH). The amino acid composition of this protein is given in Table 1.

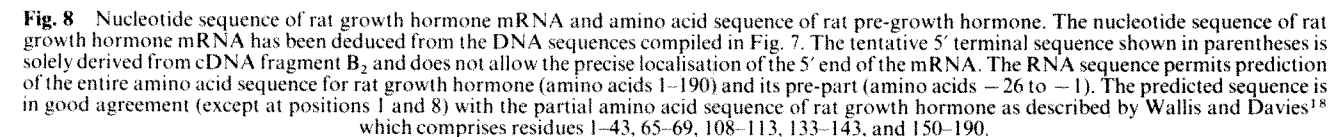
The signal peptide of pre-GH (26 amino acids, MW 2,846) has a high content of hydrophobic amino acids (17 non-polar residues,

**Table 1** Amino acid composition and codon choices of rat pre-growth hormone

Phe	UUU	6		Ser	UCU	0	(1)	Tyr	UAU	4		Cys	UGU	2	
	UUC	6	(1)		UCC	3			UAC	3			UGC	2	(1)
Leu	UUA	1			UCA	2		Ter	UAA	0		Ter	UGA	0	
	UUG	2			UCG	2			UAG	1		Trp	UGG	1	(2)
Leu	CUU	1		Pro	CCU	1	(1)	His	CAU	0		Arg	CGU	2	
	CUC	6	(2)		CCC	5	(1)		CAC	3			CGC	6	
	CUA	0			CCA	1		Gln	CAA	1	(1)		CGA	1	
	CUG	15	(4)		CCG	0			CAG	12	(1)		CGG	1	
Ile	AUU	3		Thr	ACU	1	(1)	Asn	AAU	2		Ser	AGU	1	
	AUC	5			ACC	7	(1)		AAC	3			AGC	6	(1)
	AUA	0			ACA	0		Lys	AAA	3		Arg	AGA	1	
Met	AUG	6	(1)		ACG	0			AAG	8			AGG	1	
Val	GUU	0		Ala	GCU	7	(3)	Asp	GAU	1		Gly	GGU	1	(1)
	GUC	2			GCC	8			GAC	9	(1)		GGC	3	
	GUA	0			GCA	1	(1)	Glu	GAA	4			GGA	1	
	GUG	2			GCG	2			GAG	10	(1)		GGG	3	

The amino acid sequence of rat pre-growth hormone has been solely predicted from the nucleotide sequence of rat growth hormone mRNA (see Fig. 8). Numbers next to codons indicate the number of amino acids using particular codons in the mature hormone, numbers in parentheses denote the extra amino acids of the pre-part. The N-terminal methionine is included. From a total of 216 codons used, 39.4% terminate in C, 34.3% in G, 18% in U, and 8.3% in A. The molecular weights of rat growth hormone and its precursor form as determined from the amino acid compositions are 2177.2 and 2462.6 respectively.





The amino acid sequence of rat GH determined solely from DNA sequences is in good agreement with the partial protein sequence for this hormone obtained by Wallis and Davies<sup>18</sup>. The

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1. Li, C. H. (ed.) *Hormonal Proteins and Peptides* 3 (Academic, New York, 1975).
2. Raiti, S. & Blizzard, R. M. *Adv. Pediatr.* **17**, 99–123 (1970).
3. Evans, H. M. & Li, C. H. *Science* **99**, 183–184 (1944).
4. Guyda, H. J. & Friesen, H. G. *Biochem. biophys. Res. Commun.* **42**, 1068–1075 (1971).
5. Wallis, M. *Biochem. J.* **125**, 54P–56P (1971).
6. Niall, H. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **68**, 866–869 (1971).
7. Bewley, T. A., Dixon, J. S. & Li, C. H. *Int. J. Peptide Prot. Res.* **4**, 281–287 (1972).
8. Li, C. H. *et al. Experientia* **24**, 1288 (1968).
9. Seeburg, P. H. *et al. Cell* **12**, 157–165 (1977).
10. Shine, J., Seeburg, P. H., Martial, J. A., Baxter, J. D. & Goodman, H. M. *Nature* **270**, 494–499 (1977).
11. Tashjian, A. H., Jr *et al. Endocrinology* **82**, 342 (1968).
12. Bancroft, F. C., Levine, L. & Tashjian, A. H., Jr *J. Cell Biol.* **43**, 432–441 (1969).
13. Martial, J. A., Baxter, J. D., Goodman, H. M. & Seeburg, P. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1816–1820 (1977).
14. Blobel, G. & Sabatini, D. D. in *Biomembranes* 2 (ed. Manson, L. A.) 193–195 (Plenum, New York, 1971).
15. Bancroft, F. C. *Expl Cell Res.* **79**, 275–278 (1973).
16. Sussman, P. M., Tushinski, R. J. & Bancroft, F. C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 29–33 (1976).
17. Blobel, G. & Dobberstein, B. *J. Cell Biol.* **67**, 835–851 (1975).
18. Wallis, M. & Davies, R. V. in *Growth Hormone and Related Peptides* (eds Pecile, A. & Muller, E. E.) 1–14 (Elsevier, New York, 1976).
19. Chantrenne, H., Burny, A. & Marbaix, G. *Prog. Nucleic Acid Res. molec. Biol.* **7**, 173–194 (1967).
20. Palmiter, R. D. *J. biol. Chem.* **247**, 6450–6459 (1972).
21. Duguid, J. R., Steiner, D. F. & Chick, W. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3539–3543 (1976).
22. Ullrich, A. *et al. Science* **196**, 1313–1319 (1977).
23. Martial, J. A., Seeburg, P. H., Guenzi, D., Goodman, H. M. & Baxter, J. D. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
24. Bancroft, F. C., Wu, G. & Zubay, G. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3646–3649 (1973).
25. Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
26. Dayhoff, M. O. *Atlas of Protein Sequence and Structure* **5**, Suppl. 2, 120–121 (National Biomedical Research Foundation, Washington, DC, 1976).
27. Baralle, F. E. *Cell* **10**, 549–558 (1977).
28. Shine, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 1473–1477 (1977).
29. Efstratiadis, A., Kafatos, F. C., Maxam, A. M. & Maniatis, T. *Cell* **7**, 279–288 (1976).
30. Sgaramella, V., van de Sande, J. H. & Khorana, H. G. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1468–1475 (1970).
31. Heynecker, H. L. *et al. Nature* **263**, 748–752 (1976).
32. Scheller, R. H. *et al. Science* **196**, 177–180 (1977).
33. Kornberg, A. *Science* **163**, 1410–1418 (1969).
34. Bolivar, F. *et al. Gene* (in the press).
35. Maniatis, T., Kee, S. G., Efstratiadis, A. & Kafatos, F. C. *Cell* **8**, 163–182 (1976).
36. Tushinski, R. J., Sussman, P. M., Yu, L. & Bancroft, F. C. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2357–2361 (1977).
37. Shine, J. & Dalgarno, L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1342–1346 (1974).
38. Steitz, J. A. & Jakes, K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4734–4738 (1975).
39. Shine, J. & Dalgarno, L. *Biochem. J.* **141**, 609–615 (1974).
40. Efstratiadis, A., Kafatos, F. C. & Maniatis, T. *Cell* **10**, 571–585 (1977).
41. Gralla, J. & Crothers, D. M. *J. molec. Biol.* **73**, 497–511 (1973).
42. Thiele, D. *et al. Molec. Biol. Rep.* **1**, 155–160 (1973).
43. Wilson, J. T. *et al. Nucleic Acids Res.* **7**, 2353–2368 (1977).
44. Proudfoot, N. J. *Cell* **10**, 559–570 (1977).
45. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211–214 (1976).
46. Brawerman, G. A. *Rev. Biochem.* **42**, 621–642 (1974).
47. Fiers, W. *et al. Nature* **260**, 500–507 (1976).
48. Sanger, F. *et al. Nature* **265**, 687–695 (1977).
49. Kirby, K. S. *Biochim. biophys. Acta* **36**, 117–124 (1959).
50. Devillers-Thiery, A., Kindt, T., Scheele, G. & Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5016–5020 (1975).
51. Chan, S. J., Keim, P. & Steiner, D. F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3539–3543 (1976).
52. Kemper, B. *et al. Biochemistry* **15**, 15–19 (1976).
53. Schechter, I. & Burnstein, Y. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1964–1968 (1976).
54. Lingappa, V. R., Devillers-Thiery, A. & Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2432–2436 (1977).
55. Fellows, R. E. & Rogol, A. D. *J. biol. Chem.* **244**, 1567–1575 (1969).
56. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
57. O'Farrell, P. Z., Huang, W. M. & Gold, L. M. *J. biol. Chem.* **248**, 5499–5501 (1973).
58. Dingman, C. W. & Peacock, A. C. *Biochemistry* **7**, 659 (1968).
59. Horiuchi, K. & Zinder, N. D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2555–2558 (1975).
60. Vogt, V. M. *Eur. J. Biochem.* **33**, 192–200 (1973).
61. Sugino, A. *et al. J. biol. Chem.* **252**, 3987–3994 (1977).
62. Seeburg, P. H. & Schaller, H. *J. molec. Biol.* **92**, 216–277 (1975).
63. Boyer, H. W. *et al. Nature new Biol.* **244**, 40–43 (1973).
64. Jackson, D. A., Symons, R. H. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2904–2909 (1972).
65. Cohen, S. N., Chang, A. C. Y., Boyer, H. W. & Helling, R. B. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3240–3244 (1973).
66. Sharp, P. A., Sugden, B. & Sambrook, J. *Biochemistry* **12**, 3055–3063 (1973).

# Construction and analysis of recombinant DNA for human chorionic somatomammotropin

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*DNA complementary to mRNA coding for the human polypeptide hormone, chorionic somatomammotropin, has been purified by specific restriction endonuclease digestion and religation before cloning into bacterial plasmids. The primary structure of a major portion of this mRNA species is deduced from the nucleotide sequence of the recombinant DNA.*

THE polypeptide hormone human chorionic somatomammotropin (HCS, placental lactogen) is secreted by the placenta in increasing amounts during pregnancy, reaching levels of the order of 1 g per day in the last trimester<sup>1</sup>. The physiological function of this hormone is still largely unknown, but it seems to act primarily on maternal metabolism, providing important nutrients of maternal origin necessary for foetal growth<sup>2</sup>.

The primary structure of HCS is very similar to that of human growth hormone (HGH); both polypeptides contain 191 amino acids and are 85% homologous in amino acid sequence<sup>3</sup>. Such homology suggests that the genes for these two hormones, together with that coding for the related pituitary hormone prolactin, have evolved from a common ancestral gene<sup>3,4</sup>. HCS, however, is secreted solely by the placenta, whereas growth hormone production is confined to the pituitary. This set of structurally related but differentially expressed genes thus forms an excellent system for investigating the structure, evolution and regulation of closely related eukaryotic genes.

In order to determine the degree of homology in the primary structure of these genes and to investigate the feasibility of

bacterial synthesis of human polypeptide hormones, we have constructed recombinant bacterial plasmids carrying DNA transcribed from HCS mRNA. We describe here the construction of these plasmids and the determination of the nucleotide sequence of the cloned DNA. Due to the stringent requirements of working with recombinant DNA from human sources<sup>5</sup> we have developed a general approach for preparing homogeneous cDNA fragments transcribed from a complex mixture of mRNAs which should be applicable to many other mRNA species. A comparison of the primary structure determined for the coding region of HCS mRNA with that reported for rat growth hormone mRNA in the accompanying paper<sup>6</sup> demonstrates the conservation of nucleotide sequences between these two mRNA species.

## Purification of DNA complementary to HCS mRNA

Human placental RNA was isolated from a caesarian section placenta as previously described<sup>7</sup> and the poly A-containing RNA separated by chromatography on oligo dT-cellulose<sup>8</sup>. The preparation was enriched for HCS mRNA by sucrose gradient sedimentation and pooling of the fractions (11–13S) which gave rise to a predominant 550 nucleotide cDNA *Hae*III fragment (see below). The major translation product of this RNA in a wheat germ cell free system is a 24,000 molecular weight (MW) polypeptide which accounts for approximately 50% of the total synthesis (results not shown). We have previously shown this polypeptide to be a precursor form of HCS by specific antibody precipitation<sup>7</sup>, suggesting that HCS mRNA constitutes about 50% of the total mRNA in this enriched preparation. The same polyadenylated RNA was used as a template for transcription into double-

stranded cDNA (see Fig. 1 legend). This cDNA was heterodisperse as judged by polyacrylamide gel electrophoresis but gave rise to a predominant 550-base pair fragment when digested with the restriction endonuclease *Hae*III (Fig. 1, lane 1). We have previously demonstrated that this fragment is derived from DNA complementary to HCS mRNA by direct nucleotide sequencing of the corresponding single-stranded cDNA fragment<sup>7</sup>. As seen from Fig. 1, this fragment represents approximately 30% of the total cDNA synthesis, in agreement with the levels of HCS mRNA predicted from *in vitro* translation of the same RNA template.

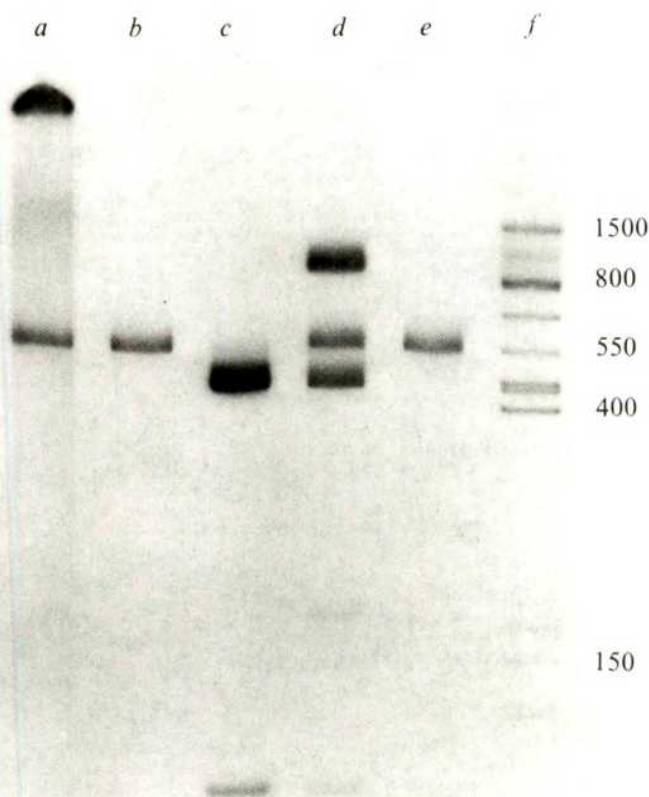
Although unique cDNA fragments generated in this manner can be isolated from most of the other fragments by gel electrophoresis and characterised by direct sequence analysis, a significant percentage of the isolated material consists of contaminating sequences. In order to purify such cDNA fragments to near homogeneity we have developed a procedure involving specific cleavage and religation of the isolated DNA fragments and have used this approach to purify HCS cDNA as shown in Figs 1 and 2. A unique DNA fragment, generated as described above from a complex mixture of DNA fragments, is first treated with alkaline phosphatase (see below), isolated by gel electrophoresis (Fig. 1, lane 2) and then cleaved into two fragments with a second restriction endonuclease. The two major cleavage products are then re-isolated following separation by gel electrophoresis. During this step most of the contaminating sequences are removed as they will remain uncleaved or will be cleaved in a different relative position (Fig. 1, lane 3). The two isolated fragments are then religated to form the original fragment. Previous treatment of the original cDNA fragment with alkaline phosphatase ensures that these two fragments are capable of ligation to each other only in the correct orientation although self-ligation to form dimers also occurs (Fig. 1, lane 4). Isolation of the original fragment, reconstructed by ligation of its constituent digestion products, results in a DNA species which is greater than 99% homogeneous as judged by restriction endonuclease digestion and quantitation of the digestion products (Fig. 2). In order to isolate HCS cDNA for molecular cloning into bacterial plasmids, we followed the above approach using *Hha*I endonuclease to cleave the 550-base pair *Hae*III fragment as described in Figs 1 and 2.

### Molecular cloning of HCS cDNA

The purified 550-base pair *Hae*III fragment was cloned into bacterial plasmids using chemically-synthesised restriction site 'linkers'<sup>11-13</sup>. The self complementary decanucleotide (5')CCGAATTCGG(3') containing the *Eco*RI restriction endonuclease recognition and cleavage site was ligated to the HCS *Hae*III fragment (previously phosphorylated using ATP and T4 polynucleotide kinase to replace the 5' phosphates removed during purification) in a molar ratio of 50:1. Figure 3 (lane 2) shows that the *Eco*RI decamers ligated both to themselves and to the HCS cDNA. Digestion of these ligation products with *Eco*RI endonuclease results in cleavage at the *Eco*RI site of the decamers giving rise to HCS cDNA with *Eco*RI cohesive ends as well as cleaved decanucleotides (Fig. 3, lane 3). As the cleaved decamers also contain *Eco*RI termini and would compete with the cDNA for ligation to the similarly cleaved plasmid, the HCS cDNA was isolated by gel electrophoresis before ligation to the cloning vector.

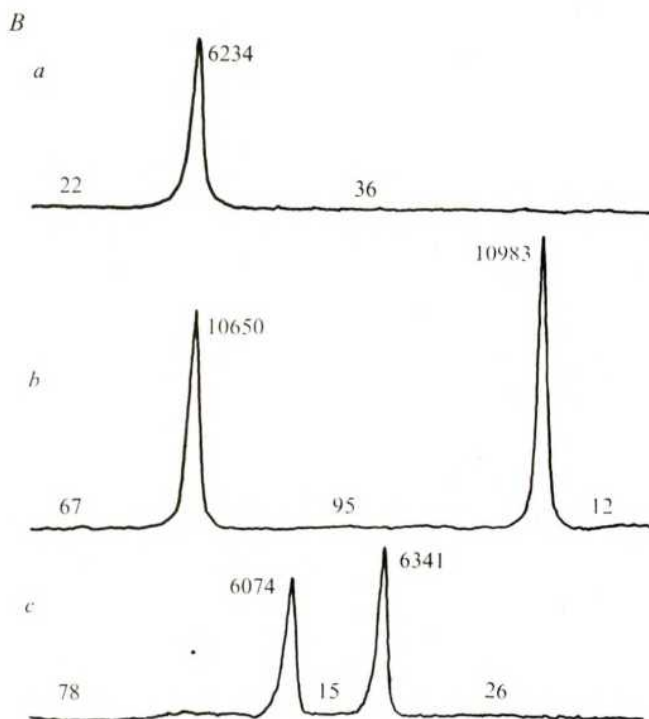
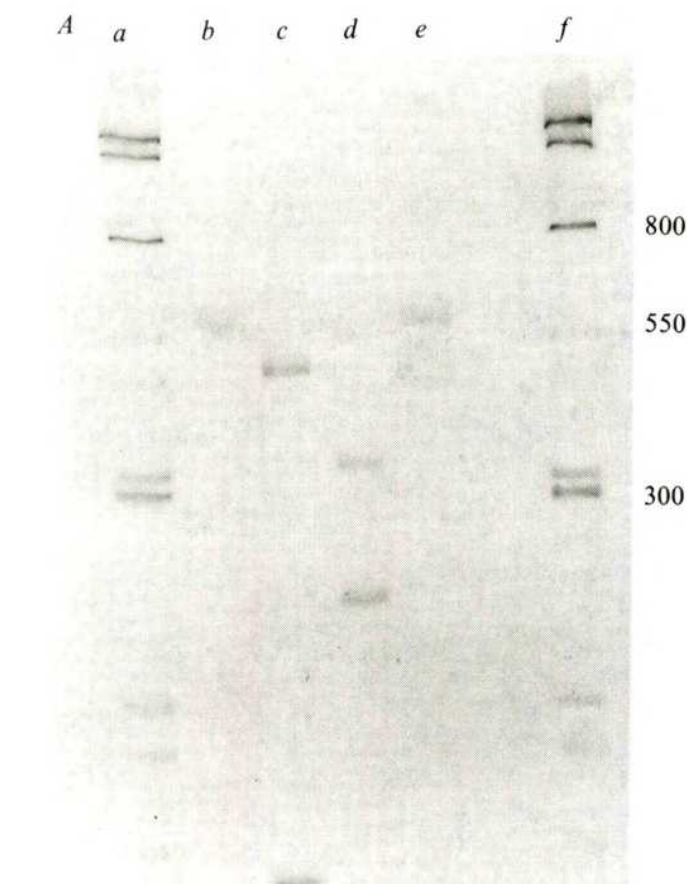
The use of this decanucleotide for the cloning of the HCS cDNA fragment has the advantage that the original *Hae*III sites are recreated at each end of the DNA fragment, allowing its isolation from the plasmid in a form identical to that of the original fragment. This is particularly useful for further studies involving the use of the cloned HCS fragment as a primer for cDNA synthesis on placental mRNA or HnRNA.

For the cloning of the HCS cDNA we used the bacterial plasmid pMB9, a  $3.5 \times 10^6$  MW molecule containing a single *Eco*RI site<sup>14</sup>. Infection of *E. coli* with pMB9 confers resistance to tetracycline. To select for recombinant plasmids we developed a method for ensuring that only plasmids containing an inserted

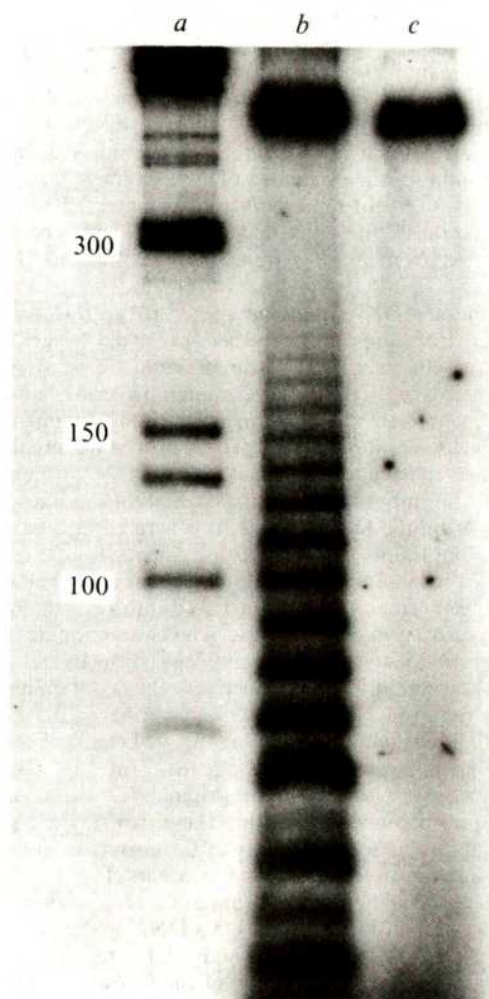


**Fig. 1** Purification of HCS cDNA. Polyadenylated placental RNA isolated as previously described<sup>7</sup> was enriched for HCS mRNA by sedimentation in a 5–20% sucrose gradient at 4 °C in the SW 27 rotor at 25,000 r.p.m. for 16 h. The 11–13S region of the gradient was pooled and 100 µg of this RNA used for the synthesis of double-stranded cDNA as described elsewhere<sup>6</sup>. DNA synthesis was stopped by extraction with phenol-chloroform and the DNA precipitated with ethanol. Digestion of the cDNA with *Hae*III endonuclease (a gift from W. Brown) was carried out in 50 µl 6 mM Tris-HCl, pH 7.5, 6 mM MgCl<sub>2</sub>, 6 mM β-mercaptoethanol with 2 units of *Hae*III at 37 °C for 2 h, after which 0.1 units of bacterial alkaline phosphatase (Worthington, BAPF) were added and digestion continued at 60 °C for 10 min. After phenol-chloroform extraction, the DNA was precipitated with ethanol, dissolved in 20 µl 10 mM Tris-HCl, pH 8, 1 mM EDTA and electrophoresed in a 6% polyacrylamide gel. The 550-base pair fragment was excised from the gel, eluted electrophoretically and digested with 4 units of *Hha*I endonuclease (New England Biolabs) at 37 °C for 2 h in 50 µl of the same buffer used for digestion with *Hae*III endonuclease. The digestion products were then separated by electrophoresis in a 6% polyacrylamide gel and the two fragments eluted electrophoretically. The eluted fragments were combined and re-ligated in a 20 µl 66 mM Tris-HCl, pH 7.6, 9 mM MgCl<sub>2</sub>, 15 mM dithiothreitol, 1 mM ATP containing 20 µg ml<sup>-1</sup> of T<sub>4</sub> DNA ligase<sup>9</sup> at 15 °C for 2 h, diluted to 200 µl with 0.1 M NaCl, extracted with phenol-chloroform and the DNA precipitated with ethanol. The ligation products were dissolved in 20 µl 10 mM Tris-HCl, pH 8, 1 mM EDTA, separated by electrophoresis in a 6% polyacrylamide gel and the 550-base pair fragment excised and eluted electrophoretically. This fragment was used in the cloning experiments. Samples from each step in the purification procedure were analysed by electrophoresis in a 6% polyacrylamide gel in 50 mM Tris-borate, pH 8, 1 mM EDTA at 100 V for 2 h. After electrophoresis the gel was dried and exposed to X-ray film (Kodak NS2T) to visualise the labelled fragments. *a*, *Hae*III digest of total placental cDNA; *b*, the 550-base pair HCS *Hae*III fragment isolated from (*a*); *c*, *Hha*I digest of the isolated *Hae*III fragment; *d*, re-ligation of the separately isolated *Hha*I digestion products; *e*, the 550-base pair fragment regenerated by ligation of its constituent *Hha*I fragments after isolation from a 6% polyacrylamide gel; *f*, <sup>32</sup>P-labelled *Hpa*II digest of ds M13 DNA used as size markers.





20  $\mu$ l 10 mM Tris-HCl, pH 8, 1 mM EDTA and subjected to electrophoresis in a 6% polyacrylamide gel. Following electrophoresis the gel was exposed to X-ray film to visualise the labelled fragments. A: a and f,  $^{32}$ P-labelled *Hae*III digest of ds M13 DNA; b and e, purified HCS cDNA *Hae*III fragment; c, *Hha*I digest of the HCS cDNA *Hae*III fragment; d, *Hpa*II digest of the HCS cDNA *Hae*III fragment. B: Scan of the autoradiogram shown in (A). a, Purified HCS cDNA *Hae*III fragment; b, *Hha*I digest of the HCS cDNA *Hae*III fragment; c, *Hpa*II digest of the HCS cDNA *Hae*III fragment. The purified HCS cDNA *Hae*III fragment was judged to be greater than 99% homogeneous by quantitation of the distribution of radioactivity in each of the two restriction endonuclease digests. Similar results were obtained by analyses of an *Xba*I digest of the purified cDNA fragment. Numbers represent c.p.m. in each fragment and in the intervening regions of the gel.



**Fig. 3** Ligation of purified HCS cDNA to *Eco*RI decamers. Purified HCS cDNA *Hae*III fragment was phosphorylated at its 5' termini (see text) using  $T_4$  polynucleotide kinase and ATP $^{10}$ . *Eco*RI decamers (5'-CCGAATTCGG-3'; a gift from R. H. Scheller) were then ligated to the fragment in a molar ratio of approximately 50:1 in 50  $\mu$ l of 66 mM Tris-HCl, pH 7.6, 9 mM MgCl $_2$ , 15 mM dithiothreitol, 1 mM ATP and 20  $\mu$ g ml $^{-1}$   $T_4$  DNA ligase $^9$ . After ligation at 4  $^{\circ}$ C for 18 h, the reaction was stopped by extraction with phenol-chloroform. The ligation products were precipitated with ethanol, redissolved in 50  $\mu$ l 100 mM NaCl, 50 mM Tris-HCl, pH 7.6, 7 mM MgCl $_2$ , and digested with 50 units *Eco*RI endonuclease at 37  $^{\circ}$ C for 2 h. Samples were analysed on an 8% polyacrylamide gel as described in the legend to Fig. 1. a,  $^{32}$ P-labelled *Hae*III digest of ds M13 DNA; b, ligation of *Eco*RI decamers to the purified HCS *Hae*III fragment; c, *Eco*RI endonuclease digest of the ligation products shown in b.

**Fig. 2** Purity of HCS cDNA used for molecular cloning. The isolated HCS cDNA *Hae*III fragment, purified as described in Fig. 1, was digested with either *Hha*I or *Hpa*II (New England Biolabs) in 50  $\mu$ l 6 mM Tris-HCl, pH 7.6, 6 mM MgCl $_2$ , 6 mM  $\beta$ -mercaptoethanol at 37  $^{\circ}$ C for 2 h and terminally labelled using [ $\gamma$ - $^{32}$ P]ATP and  $T_4$  polynucleotide kinase $^{10}$ . The labelled digestion mix was extracted with phenol-chloroform and the DNA separated from unincorporated [ $\gamma$ - $^{32}$ P]ATP by chromatography on Sephadex G-50. The labelled fragments were precipitated with ethanol, resuspended in

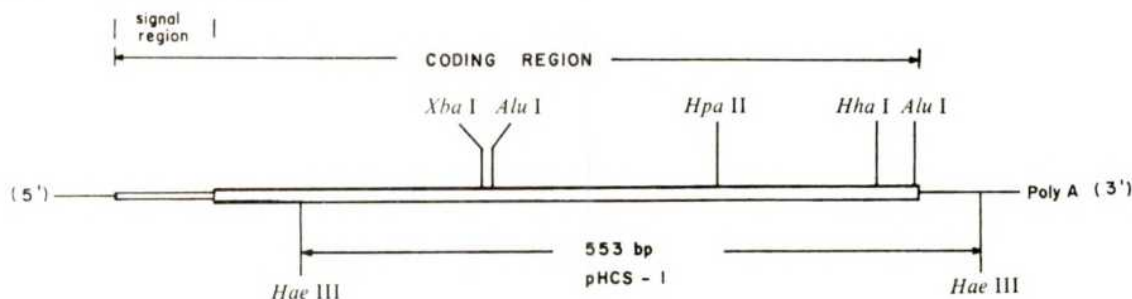


Fig. 4 Schematic representation of HCS mRNA showing restriction endonuclease cleavage sites in the corresponding cDNA. Only those restriction sites used in the present analysis are shown.

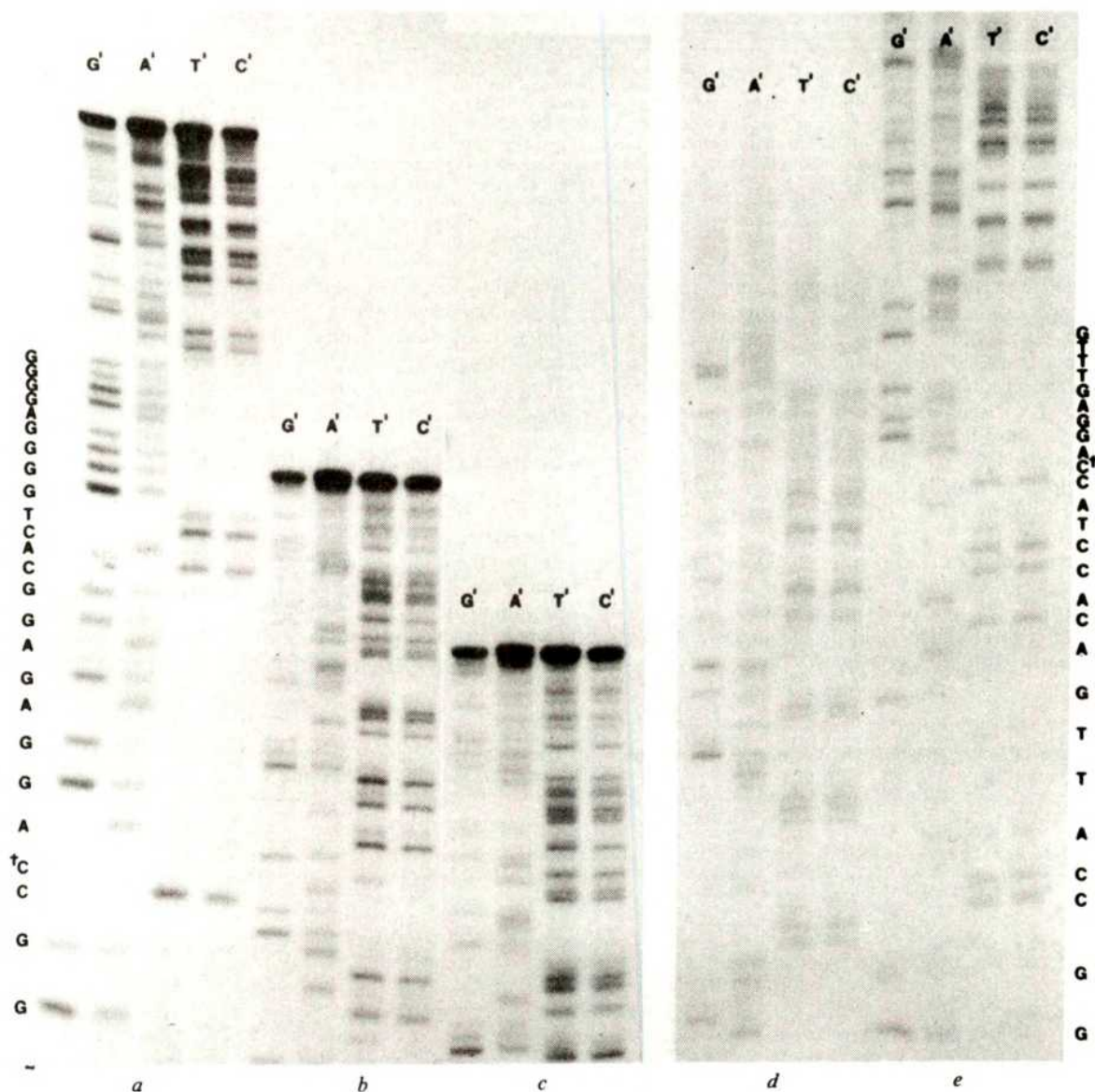
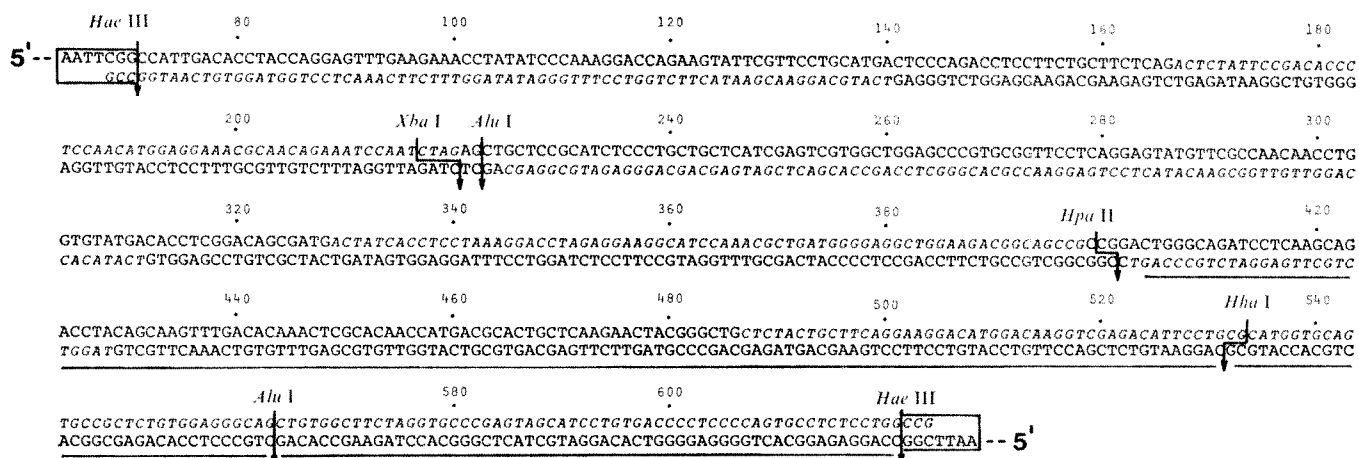


Fig. 5 Autoradiograms of 20% acrylamide-7 M urea gels used for sequence analysis of the cloned HCS cDNA fragment. The 550-base pair fragment released from pHCS-1 by *Eco*RI digestion was isolated by electrophoresis in a 6% polyacrylamide gel and the 5' termini labelled using [ $\gamma$ - $^{32}$ P]ATP and  $T_4$  polynucleotide kinase<sup>10</sup>. The labelled DNA was then incubated in 50  $\mu$ l 6 mM Tris-HCl, pH 7.6, 6 mM MgCl<sub>2</sub>, 6 mM  $\beta$ -mercaptoethanol containing 5 units of *Hpa*II at 37 °C for 2 h. The reaction was stopped by extraction with phenol-chloroform and the DNA precipitated with ethanol. The digestion products were dissolved in 50  $\mu$ l 10 mM Tris-HCl, pH 8, 1 mM EDTA, subjected to electrophoresis in an 8% polyacrylamide gel and the two labelled fragments eluted electrophoretically. Each fragment was then subjected to the four base-specific cleavage reactions described by Maxam and Gilbert<sup>15</sup> and the cleavage products analysed on a 20% acrylamide-7 M urea gel [40  $\times$  40  $\times$  0.2 cm] run at 1,000 V for 36 h. To visualise the terminally labelled fragments the gel was exposed to X-ray film (Kodak NS-54T) at -20 °C for 2 d. a, b, c, The 3'-terminal *Eco*RI-*Hpa*II fragment. Samples were loaded on the gel at 12 h intervals in the order c-b-a. d, e, The 5'-terminal *Eco*RI-*Hpa*II fragment. Sample (e) was loaded 12 h after d. G', A', T', C' refer to the respective base-specific cleavages as described by Maxam and Gilbert<sup>15</sup>. The nucleotide sequence can be read directly from the autoradiogram as shown for the 5'-terminal sequence of each fragment. \*The second C residue in the two sequences CCAGG is not seen on the gel as this is methylated in *E. coli* X1776 and hence is not cleaved during the chemical modification and cleavage reactions. Such a methylation does not affect *Hae*III cleavage at one of these sites (GGCCAGG) although it does prevent *Eco*RII cleavage at these sequences and at the CCTGG sequence found elsewhere in the cloned HCS DNA.





**Fig. 6** Nucleotide sequence of cloned HCS cDNA. The nucleotide sequence of restriction endonuclease generated fragments was determined as described in Fig. 5 legend. Only those sequences determined directly are shown in bold type. The complementary sequences are shown in italics and sequences derived from the *Eco*RI linkers are boxed. The cloned sequences were obtained by digestion of the *Eco*RI-generated fragment of pHCS-1 with either *Alu*I, *Hpa*II or *Hha*I (Fig. 4), 5'-terminal labelling of the fragments using [ $\gamma$ - $^{32}$ P]ATP and  $T_4$  polynucleotide kinase and separation of individually labelled fragments by either strand separation<sup>15</sup> (for the 5'-terminal *Alu*I fragment) or a second restriction endonuclease cleavage. The sequence is underlined where we have previously determined it directly from cDNA<sup>7</sup>. Nucleotides are numbered from the first nucleotide in the codon specifying amino acid number one (Val) in HCS<sup>3</sup>.

DNA fragment were capable of transformation. This was necessary as incorporation of DNA into the *Eco*RI site of pMB9 does not affect tetracycline resistance or any other known property of the plasmid resulting in the absence of any phenotypic difference between recombinant and normal plasmids. We therefore first treated *Eco*RI-cut pMB9 with alkaline phosphatase. This removes the 5' phosphates from the *Eco*RI-generated ends of the plasmid and prevents self-ligation of the plasmid DNA, ensuring that circle formation (and hence transformation) is dependent on the insertion of a DNA fragment containing 5' phosphorylated termini<sup>13</sup>.

The mixture containing recombinant HCS cDNA-pMB9 was used to transform the EK2 host *E. coli* X1776 as described in the accompanying article, and transformants selected by growth on medium containing tetracycline 20  $\mu$ g ml<sup>-1</sup> (work done in P3 facility in compliance with NIH guidelines). Four transformants

were obtained, all of which contained a 550-base pair insertion which was released from the plasmid DNA by either *Eco*RI or *Hae*III endonuclease digestion (see above). Furthermore, the restriction pattern of each of the four cloned DNA fragments was identical to that of the HCS cDNA fragment when cleaved with the endonucleases *Xba*I, *Hpa*II, *Alu*I and *Hha*I (see Fig. 4).

## Sequence analysis of cloned DNA

Plasmid DNA prepared from one of the clones (pHCS-1) was cleaved with *Eco*RI endonuclease and the 550-base pair insertion isolated from linear pMB9 by electrophoresis in a 6% polyacrylamide gel and subjected to DNA sequence analysis using the procedure of Maxam and Gilbert<sup>15</sup> as shown in Fig. 5. The complete nucleotide sequence of the *Eco*RI-generated fragment was determined by separate sequence analysis of the fragments

**Fig. 7** Primary structure of HCS mRNA. The nucleotide sequence of HCS mRNA was deduced from the DNA sequence determined from the cloned fragment in pHCS-1 (as shown in Fig. 6). Numbers above the sequence indicate the amino acid number from the known sequence of HCS<sup>3</sup>. The large palindrome in the 3'-untranslated region is underlined with an arrow indicating its centre of symmetry.



**Fig. 9** Conservation of nucleotide sequences in the coding region of HCS and rat growth hormone mRNAs. Two portions of the mRNA coding region of HCS (amino acids 77–96 and 128–147) and rat growth hormone (RGH) (76–95 and 126–145) are shown where the homology in amino acid sequence is high. Silent nucleotide changes (which do not result in an amino acid change) are boxed.

HCS----	Arg	Ile	Ser	Leu	Leu	Leu	Ile	Glu	Ser	Trp	Leu	Glu	Pro	Val	Arg	Phe	Leu	Arg	Ser	Met
	CGC	AUC	UCG	CUG	CUG	CUC	AUC	GAG	UCG	UGG	CUG	GAG	CCC	GUG	CGG	UUC	CUC	AGG	AGU	AUG
RGH----	CGC	UUC	UCG	CUG	CUG	CUC	AUC	CAG	UCG	UGG	CUG	GGG	CCC	GUG	CAG	UUU	CUC	AGC	AGG	AUC
	Arg	Phe	Ser	Leu	Leu	Leu	Ile	Gln	Ser	Trp	Leu	Gly	Pro	Val	Gln	Phe	Leu	Ser	Arg	Ile
HCS----	Leu	Glu	Asp	Gly	Ser	Arg	Arg	Thr	Gly	Gln	Ile	Leu	Lys	Gln	Thr	Tyr	Ser	Lys	Phe	Asp
	CUG	GAA	GAC	GGC	AGC	CGC	CGG	ACU	GGG	CAG	AUC	CUC	AAG	CAG	ACC	UAC	AGC	AAG	UUU	GAC
RGH----	CUG	GAA	GAC	GGC	AGC	CCC	CCU	AUU	GGG	CAG	AUC	CUC	AAG	CAG	ACC	UAC	GAC	AAG	UUU	GAC
	Leu	Glu	Asp	Gly	Ser	Pro	Arg	Ile	Gly	Gln	Ile	Leu	Lys	Gln	Thr	Tyr	Asp	Lys	Phe	Asp

produced by *AluI*, *HhaI* or *HpaII* endonuclease cleavage of the DNA (Fig. 6). By comparison with the known amino acid sequence of HCS, the 557 nucleotide sequence shown in Fig. 7 represents that portion of the coding region of HCS mRNA from amino acids 24–191, plus 50 nucleotides of the 3'-untranslated region. The amino acid sequence predicted from the nucleotide sequence is identical with that previously published<sup>3</sup> confirming the validity of amino acid sequence determination by nucleotide sequence analysis.

The fidelity of cDNA cloning in bacterial plasmids is seen from the presence of identical sequences in the cloned DNA and those previously determined directly by cDNA sequencing<sup>7</sup>. This is also shown in the accompanying paper on rat growth hormone mRNA<sup>6</sup> and demonstrates the validity of using recombinant DNA for structural studies of eukaryotic genes and will allow examination of the fidelity of expression of these genes in bacteria.

## Primary structure of HCS mRNA

An analysis of the coding region of HCS mRNA indicates that codon utilisation is non-random with certain strongly preferred codon choices for some of the amino acids (Fig. 8). A very similar pattern of codon utilisation is seen in rat growth hormone mRNA<sup>6</sup> which is reflected in the conservation of sequences found between these two mRNA species (see below). Preferential codon utilisation, which is clearly different from that seen in bacteriophage and SV40 mRNAs<sup>16–18</sup>, has also been previously observed

Phe	UUU	2	Ser	UCU	2	Tyr	UAU	4	Cys	UGU	1
	UUC	8		UCC	5		UAC	4		UGC	3
Leu	UUA	–		UCA	1	Term.	UAA	–	Term.	UAG	–
	UUG	–		UCG	4		UAG	1	Trp	UGG	1
Leu	CUU	–	Pro	CCU	–	His	CAU	2	Arg	CGU	–
	CUC	7		CCC	2		CAC	2		CGC	4
	CUA	3		CCA	1	Gln	CAA	2		CGA	–
	CUG	11		CCG	1		CAG	7		CGG	2
Ile	AUU	2	Thr	ACU	1	Asn	AAU	1	Ser	AGU	1
	AUC	5		ACC	5		AAC	4		AGC	4
	AUA	–		ACA	3	Lys	AAA	1	Arg	AGA	–
Met	AUG	5		ACG	2		AAG	8		AGG	3
Val	GUU	–	Ala	GCU	–	Asp	GAU	1	Gly	GGU	–
	GUC	1		GCC	2		GAC	13		GGC	4
	GUA	–		GCA	1	Glu	GAA	5		GGA	–
	GUG	4		GCG	–		GAG	8		GGG	3

**Fig. 8** Codon utilisation in HCS mRNA. Term, termination signal.

in rabbit  $\beta$ -globin mRNA<sup>19</sup> and rat insulin mRNA<sup>13</sup>. The use of certain codons (for example, CUC and CUG for Leu, GAC for Asp, AAG for Lys) seems to be favoured in all eukaryotic cellular mRNAs examined to date, while others are apparently strongly selected against (for example, UUA for Leu, AUA for Ile, GUA for Val). Such codon selection reflects the apparent preference for C or G in the third position of eukaryotic codons. In the coding region of HCS mRNA, 75 codons (46%) terminate in C, 53 (33%) in G (excluding AUG and UGG, which only have one codon), but only 17 (10%) end in U and 17 in A (10%) (Fig. 8).

Comparison of the coding regions of HCS and rat growth hormone mRNA demonstrates the conservation of nucleotide sequences between these two mRNA species with very few 'silent'

codon changes (Fig. 9), supporting the suggestion that the structural genes for chorionic somatomammotropin and growth hormone have evolved by duplication of a common ancestral gene. Such sequence conservation between related mRNAs from diverse organisms is likely to result from several factors, including selection of a favoured mRNA structure and the availability of specific isoaccepting tRNAs.

The 3'-untranslated region of HCS mRNA is characterised by a large palindromic sequence (5'-GUGACCCCUCCCC-AGUG-3') previously seen from direct sequence analysis of cDNA fragments<sup>7</sup>. In contrast to the coding region, most of the 3'-untranslated region of rat growth hormone mRNA shows very little homology with the region determined in HCS mRNA although a somewhat similar palindromic-type sequence containing oligo C tracts (5'-UCCCCCGUUAACCCCU-3') is found in a corresponding position in rat growth hormone mRNA<sup>6</sup>. Other palindromic sequences are also present in a similar position in the 3'-untranslated region of human  $\alpha$  globin mRNA (5'-UCCUCCCCUCCU-3')<sup>20</sup> and human  $\beta$ -globin mRNA (5'-GGGGGAUUAUGAAGGG-3')<sup>21</sup>. The function of such symmetrical sequences is not known, but they may represent recognition sites for specific protein-nucleic acid interactions involved in the regulation of transcription, translation or processing of the mRNA.

Amplification in bacterial plasmids of HCS gene sequences provides a probe to study regulation of this gene (and the closely related growth hormone gene) in normal and pathological states. The fidelity shown in the replication of these sequences in bacteria will also allow investigation of the expression of eukaryotic structural gene sequences when linked to bacterial promoters in a manner defined at the molecular level.

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- Pecile, A. & Muller, E. E. (eds) *Growth Hormone and Related Peptides* (Elsevier, New York 1976).
- Grumbach, M. M., Kaplan, S. L., Sciarra, J. J. & Burr, I. M. *Ann. N.Y. Acad. Sci.* **148**, 501–506 (1968).
- Niall, H. D., Hogan, M. L., Sauer, R., Rosenblum, I. Y. & Greenwood, F. C. *Proc. natn. Acad. Sci. U.S.A.* **68**, 866–869 (1971).
- Bewley, T. A., Dixon, J. S. & Li, C. H. *Int. J. Peptide Prot. Res.* **4**, 281–287 (1972).
- Recombinant DNA Research Guidelines*, National Institutes of Health Federal Register **41**, No. 131, 27901–27943 (1976).
- Seeburg, P. H., Shine, J., Martial, J. A., Baxter, J. D. & Goodman, H. M. *Nature* **270**, 486–494 (1977).
- Seeburg, P. H., *et al.* *Cell* **12**, 157–165 (1977).
- Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
- Sugino, A. *et al.* *J. biol. Chem.* **252**, 3987–3994 (1977).
- Lillehaug, J. R. & Kleppe, K. *Biochemistry* **14**, 1225–1229 (1975).
- Heyneker, H. L. *et al.* *Nature* **263**, 748–752 (1976).
- Scheller, R. H., Dickerson, R. E., Boyer, H. W., Riggs, A. D. & Itakura, K. *Science* **196**, 177–180 (1977).
- Ullrich, A. *et al.* *Science* **196**, 1313–1319 (1977).
- Bolivar, F., Rodriguez, R., Betlach, M. C. & Boyer, H. W. *Gene* (in the press).
- Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
- Fiers, W. *et al.* *Nature* **260**, 500–507 (1976).
- Sanger, F. *et al.* *Nature* **265**, 687–695 (1977).
- Pan, J., Reddy, V. B., Thimmappaya, B. & Weissman, S. M. *Nucleic Acids Res.* **4**, 2539–2548 (1977).
- Elfratiadis, A., Kafatos, F. C. & Maniatis, T. *Cell* **10**, 571–585 (1977).
- Wilson, J. T., de Riel, J. K., Forget, B. G., Marotta, C. A. & Weissman, S. M. *Nucleic Acids Res.* **7**, 2353–2368 (1977).
- Proudfoot, N. J. *Cell* **10**, 559–570 (1977).

# letters to nature

## Black holes in closed universes

BLACK holes are now the subject matter of at least half the papers in general relativity. These papers rest on a foundation of sand, for black holes have been successfully defined only in asymptotically flat spacetimes, and no one really believes that the Universe is asymptotically flat. I shall provide a firm basis for black hole physics in the present paper; I shall extend the concept of 'black hole' to arbitrary stably causal spacetimes by essentially defining a black hole to be that object which contains all the 'small' trapped surfaces. As for most astrophysical applications black hole surfaces are located approximately by the outermost trapped surface boundary, this new definition allows results which depend only on the local behaviour of black holes in asymptotically flat spacetimes to be extended (approximately) to closed universes. Results which depend on the global behaviour of black holes cannot in general be extended to closed universes. For example, it is shown that the black hole area theorem—the statement that black holes never decrease their cross-sectional area—cannot be extended to closed universes. The new concept of black hole yields a purely geometrical definition of time direction in closed universes. My notation will be that of ref. 1.

**Definition:** a black hole is the closure of the topologically smallest future set  $I^+$  such that (a)  $I^+$  contains all non-cosmological trapped surfaces; (b) the boundary of  $I^+$  is generated by null geodesic segments which are boundary generators of TIPs.

(In the topological sense, a set  $A_i$  is the smallest member of  $\{A_i\}$  if  $A_i \subseteq A_j$  for all  $i, j$ .) A black hole as defined above is a 4-dimensional object. A 3-dimensional black hole—a concept useful for discussing black hole collisions—can be obtained by intersecting the black hole defined above with a partial Cauchy surface. This is analogous to the procedure used in asymptotically flat spacetimes to obtain a 3-dimensional black hole (ref. 1, p 315).

The distinction between cosmological and non-cosmological trapped surfaces can be clarified by comparing the spherical trapped surfaces in Schwarzschild spacetime with the spherical trapped surfaces appearing in the contracting phase of a closed Friedmann universe<sup>2</sup>. In both cases the boundary of the trapped surface region  $T$  is a marginally trapped surface  $\partial T$  with  ${}_1\chi_{ab}g^{ab} = 0$  and  ${}_2\chi_{ab}g^{ab} < 0$ . (That is, one family of null geodesics orthogonal to  $\partial T$  has zero divergence at  $\partial T$ , while the other family has negative divergence at  $\partial T$ .  ${}_1\chi_{ab}$  is a null second fundamental form of  $\partial T$ .) In the Schwarzschild case the family with  ${}_1\chi_{ab}g^{ab} < 0$  points toward the trapped surfaces defining  $\partial T$ , while in the Friedmann case the  ${}_1\chi_{ab}g^{ab} < 0$  family points away from the trapped surfaces. I propose to make this distinction general.

**Definition:** for any stably casual spacetime  $(M, g)$ , let  $\partial T$  be a marginally trapped surface such that  $\partial T$  is part of the boundary of a continuous set  $T$  of acausally related trapped surfaces, and  ${}_1\chi_{ab}g^{ab} < 0$ ,  ${}_2\chi_{ab}g^{ab} = 0$ . If the family of null geodesics defined by the form  ${}_1\chi_{ab}$  point in the direction of the trapped surfaces  $T$ , then any element of  $T$  together with any trapped surface constructed by continuously deforming an element of  $T$  so that each element of the deformation is a trapped surface and is acausal with respect to all elements of  $T$ , will be called a non-cosmological trapped surface. Trapped surfaces in  $(M, g)$  which cannot be generated by the above procedure will be considered cosmological.

Recall (ref. 1, p 315) that a black hole is defined in an asymptotically flat spacetime  $(M, g)$  to be set  $M - J^-(I^+) \equiv B$ . It is known that this set contains all trapped surfaces (ref. 1,

p 311), and that its boundary is generated by the boundary generators of TIPs. It can be shown that if  $B$  arises to the future of a Cauchy surface and approaches a Kerr–Newman black hole limit, then the new definition is equivalent to the old. The details of this result will be published elsewhere.

John Wheeler is the most ardent proponent of the one-cycle closed universe<sup>3</sup>. I shall make his conception of cosmology precise by defining a Wheeler Universe to be a globally hyperbolic spacetime which is closed in space (the Cauchy surfaces are compact), and closed in time (all inextendible null geodesics are past and future incomplete and  $d(M, M)$  is finite). It is generally believed<sup>4</sup> that in the physically realistic case, an incomplete causal geodesic terminates in a singularity which destroys everything which enters it. This type of singularity I have termed<sup>5</sup> a 'strong curvature singularity'—more precisely, a point  $p$  on the b-boundary is said to be such a singularity if for every causal geodesic  $\lambda(t)$  which intersects  $p$ , all linearly independent vorticity-free Jacobi fields along  $\lambda(t)$  which are normal to the tangent vector of  $\lambda(t)$  define a volume (or area) element which vanishes as  $\lambda$  approaches  $p$ .

Thus it is expected that a physically realistic closed universe would be a Wheeler universe which begins and ends in strong curvature singularities. I will now show that black holes in physically realistic closed universes can violate the black hole area theorem.

**Theorem:** let  $(M, g)$  be a Wheeler universe for which all points on the b-boundary are strong curvature singularities. Let  $B$  be a black hole such that the null geodesic generators of  $\partial B$  all have a past endpoint at the same point  $p \in M$ . If the null convergence condition holds, then  $B$  does not satisfy the black hole area theorem.

**Proof:** there exists a Cauchy surface  $S_1$  with  $p \ll S_1$  and  $J^+(p) \cap S_1$  compact. Since all generators of  $\partial B$  intersect  $p$ , and since  $\partial B$  and  $J^+(p)$  are both closed and without boundary, we must have  $\partial B = J^+(p)$ . Hence  $\partial B \cap S_1$  is compact. The divergence  $\theta$  of the null geodesic generators of  $\partial B$  varies continuously on  $\partial B \cap S_1$ . Furthermore, since  $\partial B$  is achronal,  $\theta$  can have the value  $-\infty$  only at the point  $p$  on any generator  $\gamma$ . Now  $\theta$  satisfies the same equation as the expansion of the area defined by the vorticity-free linearly independent Jacobi fields along  $\gamma$ . Hence  $\theta$  approaches  $-\infty$  as the future limit of the affine parameter is approached, since  $\gamma$  terminates in a strong curvature singularity and the area defined by linearly independent Jacobi fields vanishes if and only if  $\theta$  approaches  $-\infty$  (ref. 1, p 100). Since the null convergence condition holds, once  $\theta$  becomes negative, it remains negative for the remainder of  $\gamma$ 's future history. Since  $(M, g)$  is globally hyperbolic,  $M = S \times R^1$ ; we can foliate  $M$  with Cauchy surfaces  $S(\tau)$ , and we can choose  $S_1 = S(\tau_1)$ . By an argument similar to that of proposition 7.24 of ref. 6, the point on  $\partial B$  at which  $\theta$  of a geodesic generator first becomes negative varies continuously with the generator  $\gamma$ . Let  $t_0$  be the affine parameter distance to the future of  $\partial B \cap S_1$  along a generator  $\gamma(t)$  at which  $\theta$  first becomes negative, with  $t_0 = 0$  if  $\theta$  is negative at  $\partial B \cap S_1$ .  $t_0$  is thus a continuous function on the compact set  $\partial B \cap S_1$ , and so it achieves its upper bound. Hence there is a Cauchy surface  $S(\tau_2)$  to the future of  $S(\tau_1)$  for which  $\theta$  is negative along every null geodesic generator of  $\partial B$  at every point in  $\partial B \cap J^+(S(\tau_2))$ . Thus the cross-sectional area of  $\partial B$  decreases, and this contradicts the black hole area theorem, which says the area never decreases.

A spherically symmetric black hole which forms by gravitational collapse in a closed Friedmann universe would satisfy the conditions of the above theorem if the matter tensor were chosen



so that the global hyperbolicity requirement and the b-boundary condition were obeyed<sup>7,8</sup>.

Many authors (for example, ref. 9) have discussed the connection between Time's Arrow and black holes. The general consensus seems to be that one obtains a preferred direction of time only in those universes for which the initial conditions allow more black holes than white holes. If this connection between entropy increase and black holes is correct, then it is possible to use the new concept of black hole to define the direction of time geometrically—no reference to the behaviour of matter is made.

**Definition:** let  $(M, g)$  be a stably causal spacetime. The future time direction of  $(M, g)$  will be the direction of time for which the volume (in the metric  $g$ ) of the region generated by all non-cosmological trapped surfaces is greatest.

This definition assumes, of course, that the volume occupied by the black holes is finite at least in one time direction. This is clearly true in Wheeler universes which begin and end in strong curvature singularities, for in this case the total volume of spacetime is finite. The relationship between the above geometrical time direction and the directions of time as defined by other, non-gravitational, physical processes will be discussed elsewhere.

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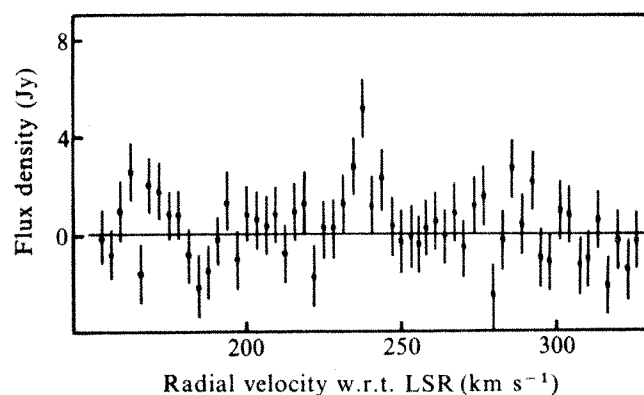
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Received 16 August; accepted 19 October 1977.

1. Hawking, S. W. & Ellis, G. F. R. *The Large Scale Structure of Spacetime* (Cambridge University Press, Cambridge, 1973).
2. Hawking, S. W. *Phys. Rev. Lett.* **15**, 689 (1965).
3. Wheeler, J. A. *The Physicist's Conception of Nature* (ed. Mehra, J.), 202 (Reidel, Dordrecht, 1973).
4. Ellis, G. F. R. & Schmidt, B. G. *J. gen. Rel. Grav.* (in the press).
5. Tipler, F. J. *Phys. Lett. A* (in the press).
6. Penrose, R. *Techniques of Differential Topology in Relativity* (Society for Industrial and Applied Mathematics, Philadelphia, 1972).
7. Einstein, A. & Strauss, E. G. *Rev. mod. Phys.* **17**, 120 (1945); **18**, 148 (1946).
8. Penrose, R. *Confrontation of Cosmological Theories with Observational Data* (ed. Longair, M. S.), 263 (Reidel, Dordrecht, 1974).
9. Davies, P. C. W. *Mon. Not. R. astr. Soc.* **177**, 179 (1977).

## Detection of H<sub>2</sub>O emission from galaxy NGC253

THE 6<sub>16</sub>–5<sub>23</sub> transition of water vapour at 22.235 GHz was observed for the first time in an external galaxy by Churchwell *et al.*<sup>1</sup> in M33 with the 100-m Effelsberg radio telescope in late 1976, after some previous unsuccessful searches<sup>2,3</sup>. We report here the second detection of H<sub>2</sub>O emission from an external galaxy, NGC253, a large edge-on spiral galaxy situated at 3.4 Mpc (ref. 4), about five times farther away than M33. Previous observations of other molecules pointed NGC253 as a good candidate for H<sub>2</sub>O emission: OH was detected by Weliachew<sup>5</sup> and confirmed by other workers<sup>6,7</sup>; CO was detected by Rickard *et al.*<sup>8</sup> and by Solomon and Zafra<sup>9</sup>, and H<sub>2</sub>CO was detected by Gardner and Whiteoak<sup>10</sup>. The time schedule of the 13.7-m Itapetinga radio telescope during 1977 May permitted a very long integration to be performed on this object, which is observable more than seven hours a day at elevation angles greater than 30°. The observations were made with a double sideband balanced mixer receiver of about 1,000 K system temperature and a 46 channel, 100 kHz resolution filter bank. Beam switching at a frequency of about 100 Hz was used, the main beam and then the 9' reference beam being pointed at the nucleus of NGC253 for alternate intervals of 1 min. The sign of the recorded signal was changed every minute by the data acquisition computer, so that possible zero-level offsets of the channels were eliminated. One-hour integrations were made with the filter bank centred alternatively



**Fig. 1** The H<sub>2</sub>O (6<sub>16</sub>–5<sub>23</sub>) spectrum of NGC253. The error bars are equal to twice the standard error of the mean obtained for each channel. The continuum flux of the nucleus of NGC253 has already been removed from the spectrum obtained from observations.

at three different frequencies in order to obtain greater velocity coverage, until a total on-source integration time of 21 h was completed at each frequency band. The data analysis program corrected the antenna temperatures for atmospheric attenuation as a function of zenith angle  $\exp(-\tau \sec z)$  and then investigated the distribution of the results of the one-hour observations for each channel, giving the standard error of the mean and rejecting data more than  $3\sigma$  different from average values; the channels were subsequently averaged two by two, simulating a 200 kHz resolution system.

The H<sub>2</sub>O spectrum of NGC253 is presented in Fig. 1, with error bars representing twice the standard error of the mean. An emission feature of  $5.2 \pm 1.2$  Jy appears at  $v = 233$  km s<sup>-1</sup>, this is possibly associated with the same emitting region which produces the 1667 MHz OH emission observed by Gardner and Whiteoak at 239 km s<sup>-1</sup> (246 km s<sup>-1</sup> heliocentric) as such small velocity differences between OH and H<sub>2</sub>O peaks are common in the sources of our galaxy, while the total velocity range due to rotation in NGC253 is larger than 200 km s<sup>-1</sup>. We have no precise information on the position of the H<sub>2</sub>O source since the 4' beam is an important fraction of the 23' × 5' galaxy. The intrinsic power of the H<sub>2</sub>O emission at 233 km s<sup>-1</sup> is about three times greater than that of W49, the strongest H<sub>2</sub>O source of our galaxy, during its maxima. The H<sub>2</sub>O emission is, however, not so anomalous as the OH emission, as Gardner and Whiteoak estimated for the OH emission at 239 km s<sup>-1</sup> an output power about two orders of magnitude greater than that of the most powerful Class I OH sources.

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Received 31 May; accepted 13 October, 1977.

1. Churchwell, E. *et al. Astr. Astrophys.* **54**, 969 (1977).
2. Dickinson, D. F. & Chaisson, E. J. *Astrophys. J.* **169**, 207 (1971).
3. Andrew, B. H., Bell, M. B., Broten, N. W. & MacLeod, J. M. *Astr. Astrophys.* **39**, 421 (1975).
4. Huchtmeier, W. *Astr. Astrophys.* **17**, 207 (1972).
5. Weliachew, L. *Astrophys. J.* **167**, L47 (1971).
6. Whiteoak, J. B. & Gardner, F. F. *Astrophys. J. Lett.* **15**, 211 (1973).
7. Gardner, F. F. & Whiteoak, J. B. *Mon. Not. R. astr. Soc.* **173**, 77p (1975).
8. Rickard, L. J., Palmer, P., Morris, M., Zuckerman, B. & Turner, B. E. *Astrophys. J.* **199**, L75 (1975).
9. Solomon, P. M. & de Zafra, R. *Astrophys. J.* **199**, L79 (1975).
10. Gardner, F. F. & Whiteoak, J. B. *Nature* **247**, 526 (1974).

## Detection of intergalactic gas in distant, rich clusters

WE have detected a reduction in intensity or 'cooling' of the cosmic microwave background as this radiation passes through several rich clusters of galaxies. These observations imply the presence of substantial quantities of hot intergalactic gas in the clusters observed. The 'cooling' effect, due to inverse Compton scattering of the microwave photons by hot intergalactic gas in the clusters, was predicted earlier by Sunyaev and Zel'dovich<sup>1</sup>. The fractional change in the intensity of the microwave background is given by

$$\frac{\Delta I}{I} = \frac{x e^x}{e^x - 1} \left[ \frac{x}{\tanh(x/2)} - 4 \right] \int_0^{\tau} \frac{k T_e}{m_e c^2} d\tau \quad (1)$$

where  $x$  is related to the observing frequency by  $x = h\nu/kT_r$ , with  $T_r$  the temperature of the microwave background, taken to be 2.7 K;  $\tau$  is the total optical depth for Thomson scattering through a cluster; and  $m_e$  and  $T_e$  are the electron mass and electron temperature of the hot gas. Gull and Northover<sup>2</sup>, observing at  $\lambda = 3$  cm, have recently reported evidence for this effect in several clusters. We have tried to confirm their work at somewhat higher sensitivity, and at a shorter wavelength. A shorter wavelength was chosen to lessen interference and confusion by radio sources in or near the clusters observed. Our results on eight clusters which are Uhuru X-ray sources will be reported elsewhere<sup>3</sup>. In general, we were able to set  $2\sigma$  limits of  $\lesssim 4 \times 10^{-4}$  K on the reduction in temperature of the cosmic background; in some cases these limits lie below the results reported by Gull and Northover. We do see statistically significant evidence for the 'cooling' effect in three clusters, however—all of which are in Abell<sup>4</sup> richness class 4. In the case of one of the three, Abell 2218, our results confirm the measurement of  $\Delta T = -1.94 \pm 0.54$  mK by Gull and Northover<sup>2</sup>. Here we call attention to the apparent strong correlation between the richness class and the detectability of the inverse

aperture and beam efficiency<sup>3</sup>, using a figure of 40%. We note that each of these values of  $\Delta T$  is larger in magnitude than any  $\Delta T$  measured for nearby, but less rich, clusters such as Coma or Abell 576.

Four corrections to the tabulated results are necessary before they can be used to draw inferences about the amount and distribution of ionised matter in these clusters. First, the observed  $\Delta T$  values are expressed in antenna temperature, and must be converted to differences in thermodynamic temperature for comparison with the 2.7 K background. The correction factor is 1.027. This reduction in the sensitivity of the results is more than off-set by an increase in the magnitude of the function of  $x$  in equation (1). With these two corrections applied, we find

$$\int_0^{\tau} \frac{k T_e}{m_e c^2} d\tau = -0.15 \Delta T$$

Next, the antenna pattern must be convolved with the surface brightness distribution of the source. The latter, of course, is model dependent. If we adopt, for instance, the adiabatic model of Sarazin and Bahcall<sup>5</sup>, with  $T_e = 10^8$  K,  $H_0 = 55$  km s<sup>-1</sup> per Mpc, and a cluster core radius of 0.25 Mpc, we find the values of the average proton number density given in Table 1. Fourth and finally, a correction should be made for the finite separation of our beams (9.0') compared to the cluster radii. For the Sarazin and Bahcall models, however, this correction can be neglected for these distant clusters.

The large size of the microwave effect and the inferred values of  $n_0$  suggest that these clusters should be luminous X-ray sources<sup>11</sup>. That they are not included in the 4U catalogue is no doubt due to their distance: all are Abell distance class 6. Our results do suggest, however, that these rich clusters—despite their distance—should be readily detected by the next generation of X-ray satellites, and that they may prove to be among the most luminous X-ray clusters.

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**Table 1** Richness class 4 clusters observed. The measured changes in temperature, with their associated standard deviations of the mean, are given. The approximate values of the proton density in the clusters are derived under the assumptions listed in the text.

Abell cluster	$\alpha_{1950}$	$\delta_{1950}$	$(\Delta T \text{ mK})$	Inferred $n_0$ ( $10^{-3} \text{ cm}^{-3}$ )
1689	13h08m58s	-01° 06.5'	$-1.06 \pm 0.46$	$5 \pm 2$
2125	15h40m26s	+66° 28.8'	$-3.10 \pm 0.34$	$14 \pm 1.5$
2218	16h35m43s	+66° 19.5'	$-2.65 \pm 0.23$	$12 \pm 1$

Compton 'cooling'. These results may be of interest to those constructing models for the intergalactic plasma in clusters<sup>5-11</sup>, especially when the results are combined with the more sensitive X-ray observations we can expect if the new HEAO satellites are successful.

Our measurements were made at  $\lambda = 9$  mm at the Cassegrain focus of the 11-m NRAO\* telescope in Tucson, Arizona, during excellent weather in April 1977. The National Radio Astronomy Observatory is operated by Associated Universities, Inc., under contract with the NSF. At the time we used them, the dual receivers had system temperatures of  $\sim 560$  K (double sideband), with  $\Delta\nu = 10^9$  Hz. The telescope beam was 3.6' full width at half-power; we employed beam switching in azimuth with a beam throw of 9.0'.

Three richness class 4 clusters were observed for periods of 5–9 h; 'cooling' of the microwave background was detected in all of them. The observed changes in temperature and the associated standard deviations of the means are shown in Table 1. All data were weighted equally in forming these means. The values shown in Table 1 have been corrected for atmospheric extinction, and for

Received 4 August; accepted 19 October 1977.

1. Sunyaev, R. A. & Zel'dovich, Ya. B. *Comments Astrophys. Space Sci.* **4**, 173 (1972).
2. Gull, S. F. & Northover, K. J. E. *Nature* **263**, 572 (1976).
3. Lake, G. & Partridge, R. B. *Astrophys. J.* (submitted).
4. Abell, G. O. *Astrophys. J. Suppl.* **3**, 211 (1958).
5. Sarazin, C. L. & Bahcall, J. N. *Astrophys. J. Suppl.* **34**, (1977).
6. Gull, S. F. & Northover, K. J. E. *Mon. Not. R. Astr. Soc.* **173**, 585 (1975).
7. Lea, S. M. *Astrophys. Lett.* **16**, 141 (1975).
8. Cavaliere, A. & Fusco-Femiano, R. *Astr. Astrophys.* **49**, 137 (1976).
9. Bahcall, J. N. & Sarazin, C. L. *Astrophys. J. Lett.* **213**, 199 (1977).
10. Gould, R. J. & Rephaeli, Y. *Compton Scattering of Microwave Background Radiation by Gas in Galaxy Clusters* (to be published).
11. Bahcall, N. A., preprint (1977).

## Radio polarisation of quasars with optical absorption spectra

CONWAY and Gilbert<sup>1</sup> have noted that the mean linear polarisation at wavelength  $\lambda$  49 cm of highly redshifted quasars is significantly less for those with optical absorption lines compared with those with none. They suggested that the cause was Faraday depolarisation in the same regions responsible for the absorption lines. Gardner and Whiteoak<sup>2</sup> pointed out similar differences between the two quasar types at  $\lambda\lambda$  11 cm and 6 cm. They regarded lower intrinsic polarisation of absorption-line quasars as a more likely explanation. Both findings were based on extremely small statistical samples. Subsequently much more data have become available at both optical and radio wavelengths. The significance of absorption-line quasars has also been enhanced. We therefore undertook a study using today's greater set of data.

**Table 1** Linear Polarisation of quasars, all  $Z_{em}$  values

		Received wavelength (cm)				
		3.7	6	11	31	49
Absorption lines	Number	21	16	24	17	16
	Median	2.8%	3.4%	2.2%	1.9%	1.1%
	Mean	$4.55 \pm 0.99\%$	$5.30 \pm 1.21\%$	$3.81 \pm 0.75\%$	$2.11 \pm 0.49\%$	$1.22 \pm 0.26\%$
No Absorption lines	All	Number	110	115	187	117
		Median	2.6%	3.1%	2.7%	1.5%
		Mean	$3.01 \pm 0.22\%$	$3.87 \pm 0.27\%$	$3.17 \pm 0.16\%$	$2.04 \pm 0.16\%$
	Equivalent numbers	Difference	$-1.54 \pm 1.01\%$	$-1.43 \pm 1.24\%$	$-0.64 \pm 0.77\%$	$-0.07 \pm 0.52\%$
		Number	16	16	26	16
		Median	1.6%	4.0%	2.7%	1.7%
	Same $Z_{em}$ values	Mean	$2.40 \pm 0.55\%$	$4.42 \pm 0.67\%$	$3.28 \pm 0.37\%$	$1.91 \pm 0.35\%$
		Difference	$-2.15 \pm 1.13\%$	$-0.88 \pm 1.38\%$	$-0.53 \pm 0.84\%$	$-0.20 \pm 0.60\%$
		Number	20	18	24	19
		Median	3.2%	4.6%	2.6%	1.0%
		Mean	$3.17 \pm 0.59\%$	$4.54 \pm 0.77\%$	$3.15 \pm 0.45\%$	$1.80 \pm 0.41\%$
		Difference	$-1.38 \pm 1.15\%$	$-0.76 \pm 1.43\%$	$-0.66 \pm 0.87\%$	$-0.31 \pm 0.64\%$

**Table 2** Linear polarisation of quasars,  $Z_{em} > 1.25$ 

		Received wavelength (cm)				
		3.7	6	11	31	49
Absorption lines	Number	16	11	18	12	10
	Median	2.8%	3.4%	1.9%	1.9%	1.1%
	Mean	$4.25 \pm 1.14\%$	$5.44 \pm 1.60\%$	$3.52 \pm 0.85\%$	$1.62 \pm 0.38\%$	$0.95 \pm 0.27\%$
No absorption lines	Number	37	31	57	30	17
	Median	2.8%	3.6%	2.3%	1.7%	0.9%
	Mean	$3.03 \pm 0.39\%$	$4.29 \pm 0.57\%$	$3.01 \pm 0.29\%$	$2.01 \pm 0.29\%$	$1.35 \pm 0.33\%$
		Difference	$-1.22 \pm 1.20\%$	$-0.51 \pm 0.90\%$	$0.39 \pm 0.48\%$	$0.40 \pm 0.43\%$

Measurements of linear polarisation at various radio wavelengths were taken from refs 3–10. Where several measurements existed for a given source at one wavelength, an average value was used. To be considered a quasar, a source had to be in the catalogue of Burbidge *et al.*<sup>11</sup>, which also served as a reference for redshifts.

We calculated the mean and the median linear polarisation for various types of quasars at wavelengths  $\lambda\lambda$  3.7, 6, 11, 31, and 49 cm. Results are summarised in Tables 1 and 2. Table 1 includes all quasars regardless of redshift; Table 2 only those with  $z_{em} > 1.25$ , the redshift criterion of the earlier studies<sup>1,2</sup>. The basic division is between those quasars having, and those not having, absorption lines. In Table 1 only, the latter are subdivided into three groups: (1) all quasars with non-absorption spectra; (2) the first 26 of them, in order of right ascension, constituting a sample comparable in size to that for the absorption-line quasars; and (3) a set consisting of quasars selected by emission redshift to match the emission redshift of an absorption-line mate. (The absorption-line quasars OQ172 and OH471, both with  $Z_{em} > 3$ , could not be matched better than  $|\Delta Z_{em}| \sim 0.8$ .)

In no case do we find a difference in mean linear polarisation between absorption and non-absorption quasars as large as two standard deviations. Only at  $\lambda$  3.7 cm do we find differences in means even slightly larger than their associated errors, and these usually in the sense that absorption-line quasars have higher mean polarisations. We conclude that the linear polarisation properties of quasars having absorption lines are indistinguishable from those not having absorption lines.

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- Conway, R. G. & Gilbert, J. A. *Nature* **226**, 332 (1970).
- Gardner, F. F. & Whiteoak, J. B. *Nature* **227**, 585 (1970).

- Wardle, J. F. C. & Kronberg, P. P. *Astrophys. J.* **194**, 249 (1974).
- Altschuler, D. R. & Wardle, J. F. C. *Mem. R. astr. Soc.* **82**, 1 (1976).
- Kronberg, P. P. & Wardle, J. F. C. *Astr. J.* (in the press).
- Wright, W. E. thesis, California Institute of Technology (1973).
- Gardner, F. F., Whiteoak, J. B. & Morris, D. *Aust. J. Phys., Astrophys. Suppl.* **35**, 1 (1975).
- Haves, P., Conway, R. G. & Stannard, D. *Mon. Not. R. astr. Soc.* **169**, 117 (1974).
- Kronberg, P. P. & Conway, R. G. *Mon. Not. R. astr. Soc.* **147**, 149 (1970).
- Conway, R. G., Gilbert, J. A., Kronberg, P. P. & Strom, R. G. *Mon. Not. R. astr. Soc.* **157**, 443 (1972).
- Burbidge, G. R., Crowne, A. H. & Smith, H. E. *Astrophys. J. Suppl.* **33**, 113 (1977).

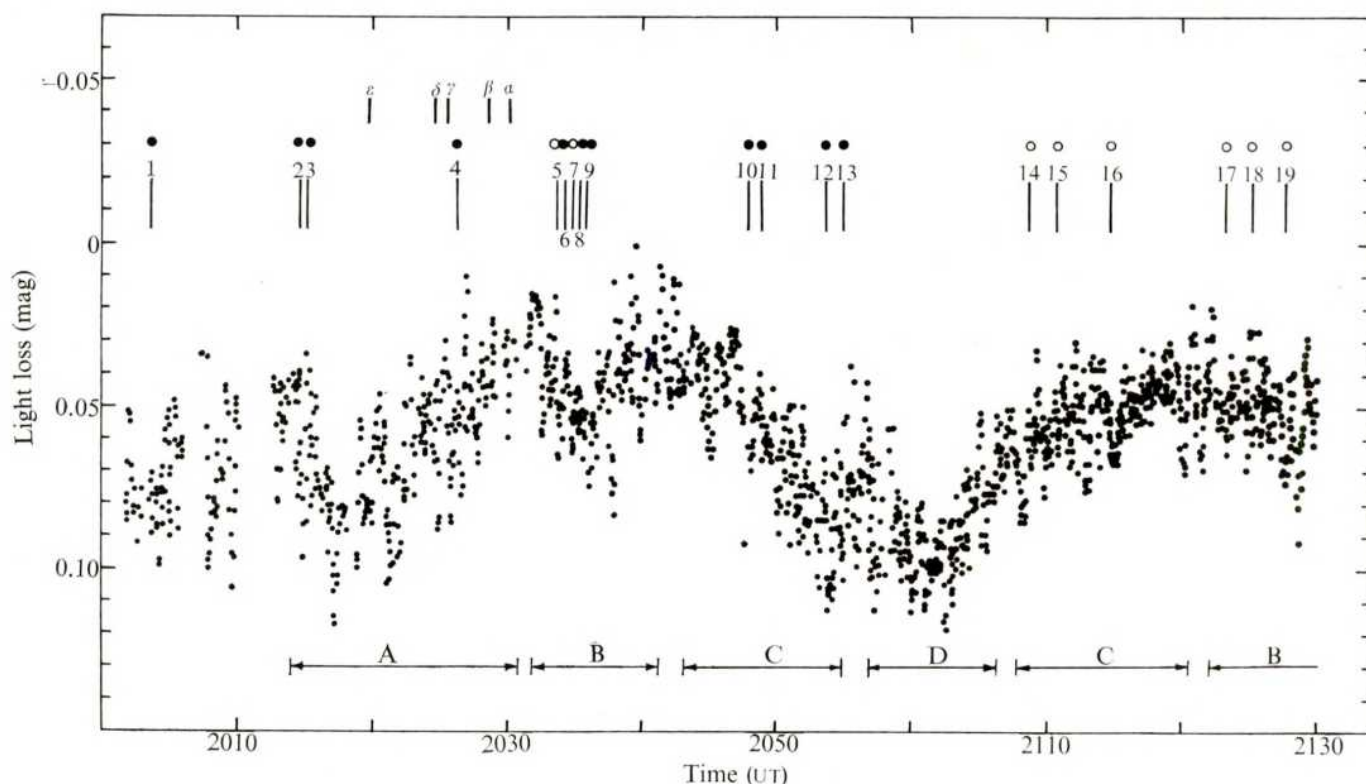
## Saturn-like ring system around Uranus

OBSERVATIONS made at Kavalur<sup>1,2</sup> of the occultation of SAO158687 by Uranus on 10 March 1977 showed, in addition to the occultation by a satellite system termed the  $\epsilon$  ring, a dimming of the light of the star close to the time when the atmosphere of the planet could have caused the phenomenon. Since then, the identities of the  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  rings have also become well established, and a theoretical interpretation of their origin<sup>3</sup> has been proposed. We have closely examined the Kavalur photoelectric record and describe here several new features of the satellite ring system of Uranus.

Our analysis consisted of scaling the photoelectric record at close intervals to study the variation in intensity. The experimental details have been described elsewhere. After careful examination we have also picked out many large spikes not reported previously which show the nature of distribution of the occulting material in the vicinity of the planet. The scaling interval is 3 s, an order of magnitude greater than the time resolution of the recording system. After correcting for atmospheric extinction and a small linear drift in the recording system, the scaled values were grouped together to give a moving average over a time interval of 15 s. Figure 1 shows these values over the entire recording period, a striking feature is the significant reduction of light of the star during the intervals 2014 to 2031, 2032 to 2041, 2042 to 2055 and 2057 to 2106 UT. We term these zones A, B, C and D respectively. An additional zone of light loss occurs during 2108 to 2120 UT.

We interpret these zones A, B, C, D as caused by occulting material distributed in an extended region around the planet.





**Fig. 1** Observed light variations at Kavalur of SAO158687 during 2000–2130 UT 10 March 1977. At the top the locations in the time coordinate where the  $\epsilon$ ,  $\delta$ ,  $\gamma$ ,  $\beta$  and  $\alpha$  rings intercept are indicated. A second row of indices numbered 1 to 19 are new spikes of short duration that we have identified on our record. The open and filled circles associated with these represent the degree of certainty in the identification of these spikes; the filled circles denote spike amplitudes that have a confidence level greater than 99.5%, while the open circles indicate visible spikes that are less certain.

Sharp discontinuities segregate these zones. A model for such a distribution of material is shown in Fig. 2 with extended ring systems A, B, C, D, similar to the ring system one sees in Saturn. Besides the occultation path, the  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$  ring condensations, the gaps in the particle distribution in the rings that appear as discontinuities, and the spike locations defining the new ring condensations that are listed in Table 1 are also marked. The distances are tentative and are on the measured basis of almanac positions of Uranus together with corrections for the relative positions of Uranus and SAO158687 according to Franz, Wasser-

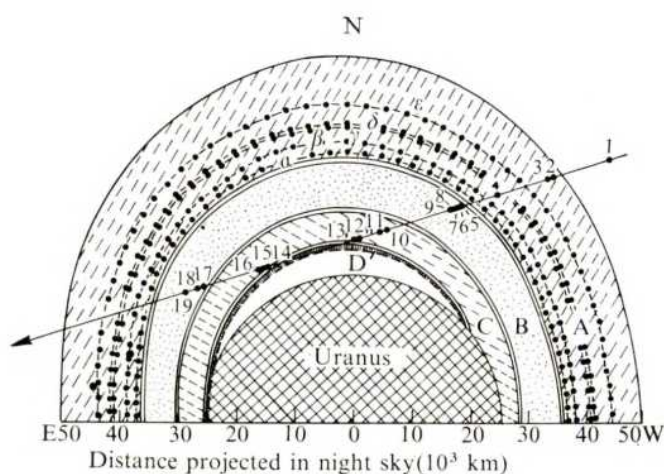
man and Seidelmann<sup>4</sup>. There is also the possibility of an inclination of the plane of the ring system to that of the equatorial plane of Uranus.

The C and D rings cause the light diminution reported earlier<sup>1,2</sup>. The D-ring effects are maximum at 2102 UT. This ring is separated from the C ring by a wide gap of  $\sim 2,000$  km. The two occasions when the light of the star encounters this gap are 2056 and 2107 UT. On the first intercept there is a near-restoration in brightness. The second event at 2107 UT is less striking. The lower boundary of the D ring is unspecified; Fig. 2 shows that it could extend nearer the planetary limb. There is considerable fine structure in both the C and D rings, as over the entire light curve of Fig. 1, portraying the light variations of the entire ring system.

Rings B and A are further away from the planet. The starlight encountered the opacity of the B ring material between 2032 and 2041 in the pre-occultation phase and again after 2122 UT. The B ring is well defined in Fig. 1, by a light loss of almost five hundredths of a magnitude. Our observations end when the light of the star is still affected by this ring on the post-occultation side. But the trend towards a reduction in intensity of starlight is clear from the light curve and substantiates our interpretation. We also note the discontinuity at 2041 and 2122 UT as caused by the gap between the B and the C rings. The light of SAO158687 shone through the A ring earlier than 2031 UT. The A–B gap is short at this epoch. The outer boundary of the A ring is poorly defined, partly because of lack of continuous record and partly because of the low altitude of Uranus at that time. Ring A contains the ring-condensations  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ . There is obscuring material even beyond the boundary we have assigned to ring A as evidenced by the spike labelled 1 in Table 1.

The ring-condensations shown by the spikes on the record are distributed over the three outer rings A, B, C. We present in Table 1 the observed times of 19 such events together with their confidence levels. In the neighbourhood of each spike over an 80-s

**Fig. 2** Diagrammatic representation of the Saturn-like ring system around Uranus. Dotted line indicates the lower boundary of the D ring.



interval of the record, we have evaluated the amplitudes of both positive and negative spikes that constitute the random noise. Assuming that this is gaussian we calculate the spike amplitude in terms of the standard deviation and read off from tables the confidence level for the spikes being of non-atmospheric origin. In Figs 1 and 2 we have listed only those spikes that are easily seen on the tracing; the lowest level of confidence of those in Table 1 is 97.9%.

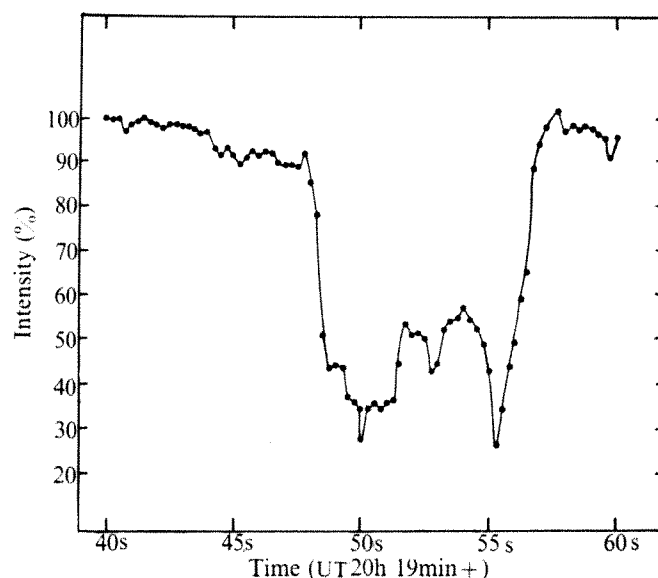
These ring-condensations seem to vary in opacity along the ring plane. As examples, we cite the spike numbers 5 and 6 which coincide with events 4 and 5 reported by Millis, Wasserman and Birch<sup>5</sup>. Both of these events have very high confidence levels of 99.67 and 99.99% respectively on the Lowell records. (We determined this value from samples of Lowell tracing provided by Wasserman.) On the other hand, these events, on our records,

**Table 1** Confidence levels for spike events

Event no.	Confidence level (%)	Event no.	Confidence level (%)
1	99.8	11	99.8
2	99.8	12	99.8
3	99.6	13	99.5
4	99.9	14	99.0
5	98.8	15	99.0
6	99.6	16	98.9
7	99.0	17	97.9
8	99.5	18	98.1
9	99.5	19	99.2
10	99.9		

have confidence levels of 98.8 and 99.6% respectively. In fact we record spikes of higher probability of occurrence than event 5 of Millis *et al.* The inference is, therefore, that longitudinal variations of opacity in the plane of the ring-condensations exist.

An exception seems to be the relatively wide ring-condensation termed  $\epsilon$ . In Fig. 3 we give a light curve of occultation by this ring



**Fig. 3** Intensity variations caused by the  $\epsilon$  ring.

from closely spaced measures of the photoelectric tracing every quarter of a second. Note that there is much fine structure in the variation of transparency that may be due to resonances associated with Titania and Oberon as postulated by Dermott and Gold<sup>3</sup>. Also of note is the close agreement between the light curve of the ring found by Millis *et al.*<sup>5</sup> and by us, even though the intercepts with the ring are made at locations several thousand kilometres apart.

Table 2 summarises the above discussion of an extended ring system about Uranus, similar to that of Saturn. By dividing the Table into the pre- and post-occultation phases and locating events by time, we avoid the uncertainties of values of distance. If most of these particles are small and abundant enough to have

**Table 2** The extended ring system of Uranus

Ring feature	Time of Crossing		Spike no.	Pre-occultation		Spike no.	Post-occultation	
	Pre-occultation	Post-occultation		Time (h min s)	Time (h min s)		Time (h min s)	Time (h min s)
Outer boundary	20 14		1	20 03 28				
$\epsilon$ ring-condensation	20 19 51		2	20 14 37				
$\delta$ ring-condensation	20 24 41		3	20 15 10				
A $\gamma$ ring-condensation	20 25 41							
$\beta$ ring-condensation	20 28 40		4	20 26 21				
$\alpha$ ring-condensation	20 30 11							
Inner boundary	20 31							
Gap A-B								
Outer boundary	20 32		5	20 33 40				
B			6	20 34 08				
			7	20 34 47	19		21 27 37	
			8	20 35 15	18		21 25 08	
			9	20 35 48	17		21 23 12	
Inner boundary	20 41	21 22						
Gap B-C								
Outer boundary	20 42	21 20	10	20 48 06				
C			11	20 48 51	16		21 16 58	
			12	00 52 52	15		21 10 40	
			13	20 54 53	14		21 08 45	
Inner boundary	20 55	21 08						
Gap C-D								
D Outer boundary	20 57	21 06						

All times in UT

caused these opacity effects, their albedo, assuming them to be similar in constitution to those in Saturn's rings, should make the presence of the extended ring system observable.

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1. Bhattacharyya, J. C. & Kuppuswamy, K. *IAU Circ.* No. 3048 (1977).
2. Bhattacharyya, J. C. & Kuppuswamy, K. *Nature* **267**, 331–332 (1977).
3. Dermott, S. F. & Gold, T. *Nature* **267**, 590–593, (1977).
4. *IAU Circ.* No. 3038 (1977).
5. Millis, R. L., Wasserman, L. H. & Birch, P. V. *Nature* **267**, 330–331, (1977).

## Why is a minor planet minor?

ALL planets and satellites are generally believed to have formed by accretion of small bodies. The craters on the Moon, Mercury and Mars give clear evidence that the accretion of small bodies was of major importance, at least during the last phase of their formation. It is very likely that collision and fragmentation of planetesimals played a key part in the accretionary stage of planets. As suggested by Hartung<sup>1</sup>, accumulation and fragmentation of planetesimals may have competed in the planetary accretion process, and a planetesimal which could survive catastrophic destruction may have become a planet. On the basis of the accretion model of planet formation, we propose a new idea in which we explain why minor planets are 'minor' and could not grow to a full-size planet. These results also substantiate Orowan's<sup>2</sup> idea that terrestrial planets accreted inhomogeneously, with iron being the first to accumulate and silicates the second.

Modes of fragmentation and accumulation of planetesimals during a collision depend on material property, impact velocity, and relative size of the impacting planetesimals. To simplify the discussion we consider only a mass ratio of projectile:target of  $10^{-2}$ . If the target is the largest planetesimal in the system and the size distribution of the planetesimals follow  $dn \propto m^{-5/3} dm$ , then the planetesimals larger than the projectile of the above mass ratio comprise about 50% of the total mass. Our numerical simulation of planetary accretion<sup>3</sup> also indicates that the collision of the planetesimals with above projectile:target mass ratio may control the evolution of the total planetesimals.

As a result of collision between two planetesimals, there are three possible fates: (1) bouncing, (2) accumulation, and (3) fragmentation or net mass loss of the target planetesimal. If the collision of two planetesimals is elastic, the two planetesimals will rebound from each other and the two will not coagulate, when the impact velocity is larger than the escape velocity of the target planetesimals. If the collision is made at a very large velocity, then the two planetesimals are broken into smaller fragments and major parts of the fragments may acquire kinetic energy large enough to escape from the two-body system. If the impact velocity is between the first (elastic) case and the second (fragmentation) case, ejecta material at the impact does not have a velocity large enough to escape from the target and it may eventually accumulate on the target planetesimals. Only this case will contribute to the net growth of the planetesimal.

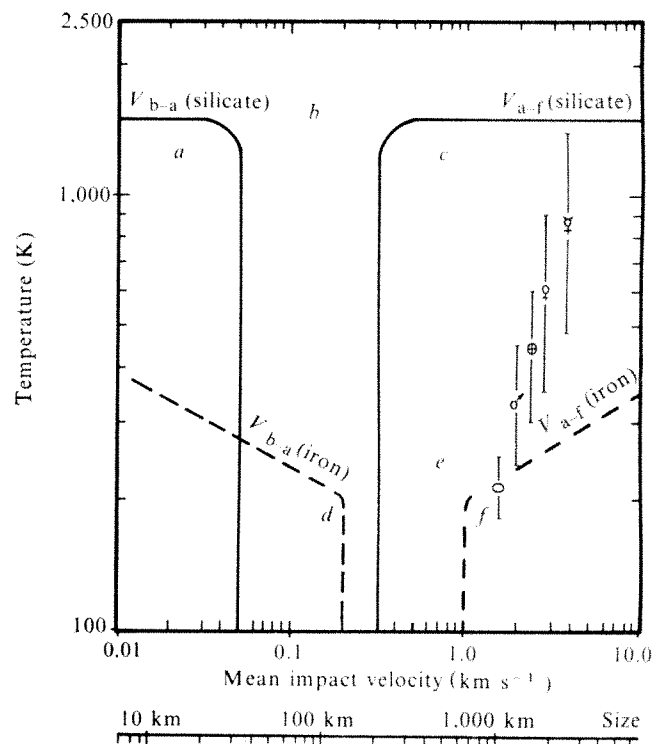
The critical impact velocity subdividing the above three cases depends on the material properties of the target and projectiles. For instance, if colliding bodies are very plastic, they are not easily disrupted but coagulate even at a rather high impact velocity. Orowan<sup>2</sup> first pointed out the important effect of the plastic (or ductile) property of iron on the planetary accretion process. He suggested that iron and

nickel are nucleating agents of the inner planets, because metallic particles are expected to stick together by 'cold welding' or 'hot welding' more easily than silicates.

It is important to note here that mechanical properties also change significantly with temperature. Iron is no longer ductile at temperatures below  $\sim 200$  K, as shown by an impact experiment with the Gibeon iron meteorite<sup>4</sup>. Many other metallurgical studies<sup>5</sup> also show that most irons undergo brittle-ductile transition at temperatures between 200 and 250 K. Therefore iron particles cannot be the nucleating agents at temperatures below 200–250 K. We propose here that this important factor determines the asteroid's evolution. Silicates become ductile only at a high temperature, however (for example, most silicate rocks would probably show the brittle-ductile transition at  $T \sim 1,500$  K at impact conditions of interest).

The critical velocity,  $V_{a-f}$ , subdividing the accumulation regime and fragmentation regime, is estimated as follows. According to Gault and Wedekind<sup>6</sup> and Fujiwara *et al.*<sup>7</sup> a kinetic energy of  $10^7$  erg  $g^{-1}$  will be enough to completely fragment a basaltic body. When the mass ratio of the projectile:target is  $10^{-2}$ , the velocity corresponding to the above energy is about  $0.3$  km  $s^{-1}$ ;  $V_{a-f}$  (silicate)  $\sim 0.3$  km  $s^{-1}$ . In the case of an iron target at brittle condition,  $V_{a-f}$  is 5 to 10 times larger than that for a silicate rock, because iron has a higher tensile strength and a higher failure (strain) limit; both of these being a factor of 2 or 3 higher than ordinary silicate rocks. Therefore the critical velocity,  $V_{a-f}$  (iron) may be around  $1$  km  $s^{-1}$  at a brittle (low temperature) condition. On the other hand, at a ductile condition,  $T > 200$  K for irons,  $T > 1,500$  K for silicate rocks,

**Fig. 1** Temperature and impact velocity conditions for bouncing, accumulation and fragmentation of planetesimals. The solid lines indicate boundaries for silicate material. The broken lines indicate boundaries for iron planetesimals. The symbols are for Mercury, Venus, Earth, Mars and Minor planets and show the estimated temperatures and mean impact velocities of planetesimals in each proto-planetary particulate ring. The scale of size corresponding to the escape velocity ( $=$  the mean impact velocity here) is shown. *a*, Stone rebound; *b*, stone accrete; *c*, stone disrupt; *d*, metal rebound; *e*, metal accrete; *f*, metal disrupt.





these materials are tougher than at a brittle condition, because most of the impact energy is absorbed for the plastic deformation of the material. Correspondingly, the critical velocity  $V_{a-f}$  at a ductile condition may become substantially higher than the above estimates. We expect, therefore, the boundaries dividing the accumulation and fragmentation regimes to be like those shown in Fig. 1.

The critical velocity,  $V_{b-a}$ , subdividing the bouncing and accumulation regimes is the minimum impact velocity which generates a stress higher than the elastic (tensile) limit,  $\sigma_e$ , of the material. A simple calculation yields the following values: assuming  $\sigma_e$  (silicate) = 0.5 kb, and  $\sigma_e$  (iron) = 5 kb,  $V_{b-a}$  (silicate)  $\sim 0.05 \text{ km s}^{-1}$ , and  $V_{b-a}$  (iron)  $\sim 0.2 \text{ km s}^{-1}$ . These estimates are consistent with impact experiments by Hartmann<sup>8</sup> for silicate rocks. The critical velocity  $V_{b-a}$  also changes with temperature, because the strength of the material changes with the temperature. The velocity is expected to become smaller as the temperature increases.

Thus we obtain a map of the bouncing accumulation, and fragmentation regimes as a function of temperature and impact velocity, shown in Fig. 1. We stress that Fig. 1 is only schematic, because the exact position of the boundaries might be affected by the shape, impact angles and other properties of the colliding planetesimals.

In Fig. 1, silicate planetesimals can grow only in the region *b*, and iron planetesimals in region *e*. Therefore, only the area superposed by both the regions *b* and *e* satisfies the condition for both iron and silicate planetesimals to grow. In other regions only one component of them (either iron or silicate) or neither of them, can grow.

The next problem is to estimate temperatures and mean impact velocities for planetesimals in a proto-planetary particulate disk. According to Lewis<sup>9</sup>, the bulk compositions of Mercury, Venus, Earth, Mars and the asteroids Ceres and Vesta are dominated by materials formed in the condensation temperatures around 1,400 K, 900 K, 600 K, 450 K and 250 K respectively. These temperatures may give upper bounds of planetesimal temperatures in each planet zone. Lower bounds on the planetesimal temperatures are probably given by the radiative-equilibrium temperature at the present planet positions. These are 480 K, 350 K, 300 K, 240 K and 180 K, for Mercury, Venus, Mars and the asteroids respectively. We tentatively assume that the temperature of planetesimals during the accretion-collision stage lies between these two limits.

It is also very difficult to estimate the impact velocities. If there are no dense gases in the terrestrial planet's region at the time of accretion, the planetesimal may acquire a relatively high velocity due to the distant and/or close encounters of other planetesimals. The mean relative velocity of planetesimals may be approximated by  $V^2 = (e^2 + i^2) v_k^2$ , where  $e$ ,  $i$ , and  $v_k$  are eccentricity, inclination and Kepler orbital velocity of each planet. If we take the mean eccentricity and inclination of the terrestrial planets ( $e = 0.08$  and  $i = 0.0$ ), the mean velocity of planetesimals in the terrestrial planet region becomes higher than  $1 \text{ km s}^{-1}$ .

The temperatures and the mean impact velocities estimated above are plotted in Fig. 1. As seen, almost all the data points lie in the region (*c+e*), where only iron-like materials can coagulate. This observation supports Orowan's idea that iron-like material is the nucleating agent in the terrestrial planets. Silicates could probably not accumulate until the iron-nucleus grew to about 1,000 km in radius. This result gives another dynamical mechanism for heterogeneous accretion of planets.

Figure 1 indicates that asteroids are in a marginal position; the asteroid condition may fall in region *f*. This indicates that iron-like planetesimals in the asteroid zone had difficulty in growing due to their brittleness at low temperature, so that iron could not be a nucleating agent in the asteroid zone. Probably the iron-like material could

not grow at all or would grow too slowly to become large enough to form a planetary nucleus. The small iron planetesimal also fails to capture enough silicate material for a full-size planet. Thus, it is very likely that the low temperature condition of the asteroids hampered their growth into iron-like planetesimals, which in turn inhibited the accumulation of silicate planetesimals. In conclusion, we propose that the brittleness of iron at temperatures below 200–250 K is the primary reason why minor planets are minor, and why minor planets could not grow to full-size planets.

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1. Hartung, J. B. *Lunar Science* 6, 337–339 (The Lunar Science Institute, Houston, 1975).
2. Orowan, E. *Nature* 222, 867 (1969).
3. Matsui, T. thesis, Univ. Tokyo (1976).
4. Remo, J. L. & Johnson, A. A. *J. geophys. Res.* 80, 3744–3748 (1975).
5. Hahn, G. T., Averbach, B., L. Owen, W. S. & Cohen, M. in *Fracture* (eds Averbach, B. L., Felback, D. K., Hahn, G. T., & Thomas, D. A.) 91–116 (MIT Press, Cambridge, 1959).
6. Gault, D. E. & Wedekind, J. A. *J. geophys. Res.* 74, 6780–6794 (1969).
7. Fujiwara, A., Kamimoto, G. & Tsukamoto, A. *Icarus* 31, 277–288 (1977).
8. Hartmann, W. K. *Lunar Science* 8, 403–405 (The Lunar Science Institute Houston, 1977).
9. Lewis, J. S. *Science* 186, 440–443 (1974).

## Kimberlites and plate-tectonics in West Africa

MESOZOIC kimberlites in West Africa are the source of most of the alluvial deposits of diamond which bolster the economies of Sierra Leone, Guinea, Liberia, Ivory Coast and Ghana. Difficulties encountered in the interpretation of geophysical, geochemical and mineralogical prospecting methods led to a structural investigation into the distribution and tectonic controls of kimberlite magmatism in eastern Sierra Leone. Here we attempt to show a correlation between the continental continuations of transform fault fracture zones and kimberlite magmatism in West Africa.

Areas of richly diamondiferous alluvial deposits, unabraded picro-ilmenite and pyrope occurrences and outcrops of kimberlite pipes and dykes indicate a broad east–north-east swathe of kimberlitic activity cutting through West Africa from Sierra Leone to northern Ghana<sup>1–4</sup>. Figure 1 shows that Mesozoic kimberlite fields within this zone of activity are frequently centred near well developed north–south brittle structures, as in eastern Sierra Leone and western Ivory Coast. In Sierra Leone, east–north-east trending kimberlite dykes cut across Archaean north–south and south-east trending structures, but parallel local east–north-east fractures confined between major north–south regional faults. Only in the coastal strip of Sierra Leone and Liberia are regional scale east–north-east structures apparent; elsewhere, they are short, disconnected structures of only local importance.

The Atlantic Ocean floor is cut by still active transform fault fracture zones which are orientated along east–north-east directions where they intersect with the coastline<sup>5,6</sup>. Two such fracture zones intersect the Sierra Leone coastline; the Guinée Fracture Zone and the Sierra Leone Fracture Zone. When traced inland their hypothetical continuations are the loci of kimberlitic intrusions, but are not major structures as in Nigeria<sup>7</sup>, being subtly incorporated into the regional structure. The Sierra Leone Frac-

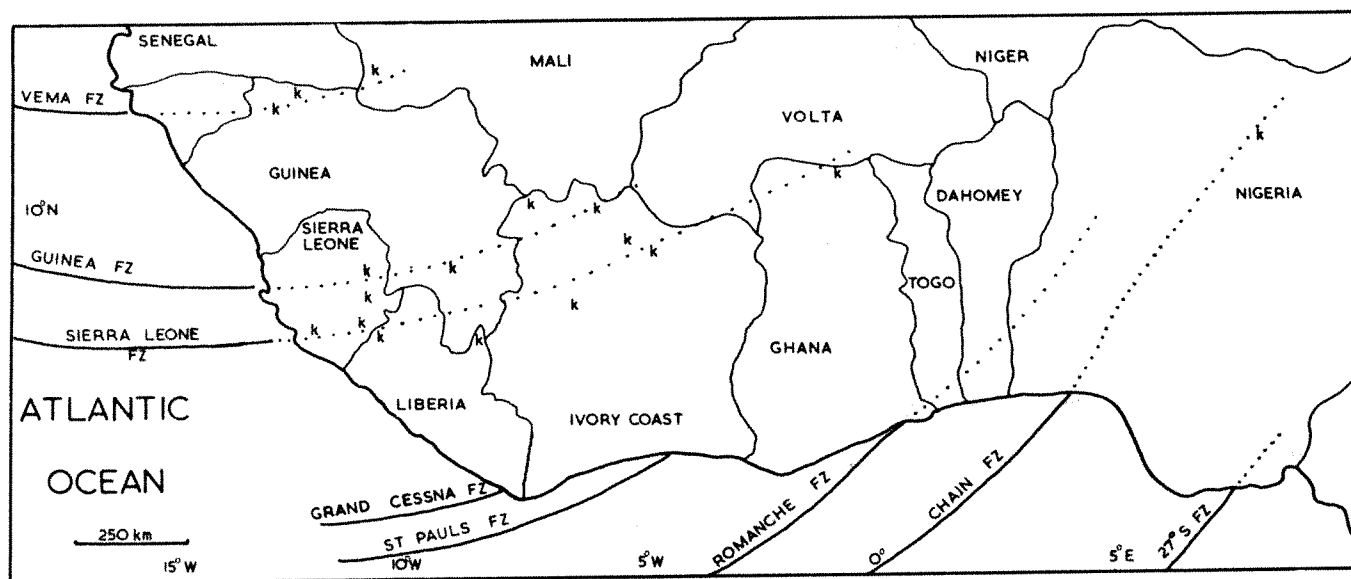


Fig. 1 West Africa, showing locations of kimberlites of probable Mesozoic age, (k); oceanic fracture zones (FZ) and their hypothetical continental continuations.

ture Zone may be traced 150 km inland as the fault-controlled estuary and course of the Sewa River, while the Guinea Fracture Zone meets the coastline at Freetown, and is recognisable as a major fault-controlled estuary along which two layered basic intrusions are developed<sup>8</sup>.

Mesozoic kimberlitic activity occurs elsewhere in West Africa, in northern Guinea and in Nigeria<sup>7</sup>. In Nigeria, kimberlite is found, as in Sierra Leone, close to the continental continuation of a major fracture zone, the Chain. In Mali<sup>4</sup>, the close association of kimberlites and Mesozoic basalts suggests a Mesozoic age for the former. In northern Guinea and Mali, the kimberlites lie on the hypothetical continental continuation of the Vema Fracture Zone. Similar relationships between magmatism and oceanic fracture zones were proposed by Marsh<sup>9</sup>. He supported the hypothesis that the continental expressions of the fracture zones were small circles about the pole of rotation of seafloor spreading. In West Africa, the pre-80 Myr spreading took place about a pole situated at 21.5°N, 14°W (ref. 10). At the time kimberlites were being intruded into the crust, the pole of rotation for the Africa-America spreading system was moving north towards its present position<sup>11</sup>. During pole migration the fracture zones and their continental expressions became activated, perhaps allowing increased penetration of kimberlitic magma into the crust. The absence of kimberlites along hypothetical continental extensions of fracture zones such as the St Pauls and Romanche may be due simply to the lack of kimberlitic magma along these structures, or to insufficient tectonism to allow its upward movement.

A relationship between kimberlites and structures associated with plate-tectonics is suggested in West Africa, and confirms a hypothesis based on south-west African data<sup>9</sup>.

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- White, R. W., Leo, G. W. *Geol. Surv. Lib. Bull.* 1 (1969).
- Knopf, D. *Les kimberlites et les roches apparentées de Côte d'Ivoire* (Sodemi, Abidjan, 1970).
- Grantham, D. R. & Allen, J. B. *Overseas Geol. Miner. Res.* 8, 5-25 (1960).
- Bardet, M. *Mém. Bur. Rech. Géol. Minier* 83 (1973).
- McMaster, R. L., Christofferson, E. & Ashraf, A. *Am. Ass. Petrol. Geol. Bull.* 59, 2161-71 (1975).

- Blundell, D. J. *Earth planet. Sci. Lett.* 31, 287-90 (1976).
- Wright, J. B. *Tectonophysics* 34, T43-47 (1976).
- Wells, M. K. *Overseas Geol. Min. Res. Bull. Suppl.* 4, 115 (1962).
- Marsh, J. S. *Earth planet. Sci. Lett.* 18, 317-323 (1973).
- Francheteau, J. *Am. Ass. Petrol. Geol. Bull.* 56, 991-1007 (1972).
- Pitman, W. C. III & Talwani, M. *Geol. Soc. Am. Bull.* 83, 619-646 (1972).

## Laminar-turbulent transition in polymer solutions

WHETHER transition from laminar to turbulent flow is delayed in dilute drag-reducing polymer solutions, unchanged, or occurs at an earlier Reynold's number has been a vexing question since research began in this area. Using the classic Reynold's dye-streak technique, Giles and Pettit<sup>1</sup> showed that pipe-flow turbulence was delayed by a factor of 2 or more by small additions of polymer. Similarly, Castro and Squire<sup>2</sup> and White and McEligot<sup>3</sup> found transition delay in small pipes from measurements of pressure fluctuations and actual drag-reduction performance. But more refined pipe-flow studies by Little *et al.*<sup>4</sup> show convincing evidence for polymer solution transition earlier than for the pure solvent and this has been confirmed by laser-Doppler anemometry<sup>5</sup>. Here we show that polymer solutions in a boundary-layer flow exhibit a transition to turbulence at a lower Reynold's number than the pure water solvent.

Following the work of Brennen<sup>6</sup>, an ogival-shaped head with an axial length of 3½ inches and a base diameter of 2.34 inches was tested in the Naval Academy free-surface water tunnel. The head shape corresponds to a source distribution, cut off sharply and supported by a long sting through which air was supplied to ventilate the cavity, and through which the cavity pressure could also be measured. By ventilation, a fully developed cavity could be supported at a Reynold's number (based on ogive length) of  $3 \times 10^5$  and a cavitation number,  $\sigma$ , of 0.5 where  $\sigma = (P_T - P_c) / \frac{1}{2} \rho U^2$  and  $P_T$  and  $P_c$  are the tunnel and cavity pressures and  $U$  is the tunnel velocity (10 ft s<sup>-1</sup>). The ventilated cavity as seen by the eye is shown in Fig. 1a; the cavity rising with respect to the water flow due to its buoyancy.

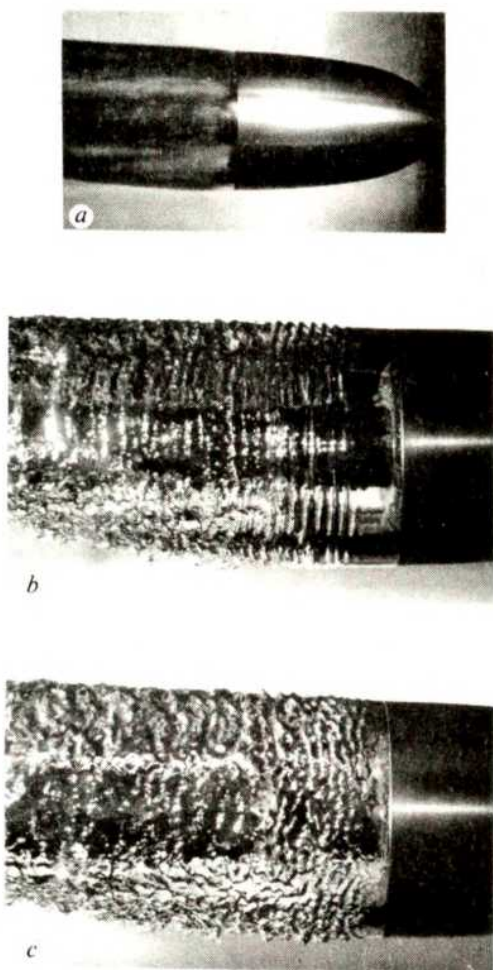
When the cavity is illuminated by a stroboscope, or photographed using a short-duration flash, the series of waves shown in Fig. 1b is readily discernible on the cavity in pure-water flow. These waves represent the cavity-flow



equivalent to the instabilities in a free shear layer in a (one-phase) wake flow and have been studied by Brennen<sup>6</sup>. Note that the flow leaving the ogive surface is laminar, and the waves are part of the laminar-turbulent transition process on the surface of the cavity.

At the same tunnel velocity and pressures, but with 25 p.p.m. solution of polyacrylamide (Calgon TRO-375) instead of water, the laminar zone in the flow leaving the ogive has disappeared as shown in Fig. 1c. A similar appearance to Fig. 1c was obtained by roughening the surface of the ogive in pure water flow (although we were careful to avoid roughening the ogive surface when using polymer solution). Thus, we conclude that the presence of polymer additives causes an earlier transition to turbulence in the boundary layer of the ogive than does the pure-water flow. A similar conclusion could be drawn from photographs of water and polymer solution jets discharging in air<sup>7</sup>, but the test geometry is not as well defined as in the present study.

At the wall shear rate involved ( $10^4 \text{ s}^{-1}$ ), the presence of the polymer has increased the water viscosity in the tunnel by about 40% (as estimated from rheograms for a polymer of similar drag-reduction effectiveness<sup>8</sup>) and thus the Reynolds number for the polymer flow has decreased to around  $2 \times 10^5$ . The effect of the polymer on transition is, hence, even more marked than indicated by the photographs.



**Fig. 1** Photographs of ogive head-form in water tunnel. (Flow is from right to left.) *a*, Overall view of body and cavity; *b*, short-duration flash ( $10 \mu\text{s}$ ) showing laminar flow leaving ogive and transition to turbulence.  $Re = 3 \times 10^5$ ,  $\sigma = 0.5$ ; *c*, same tunnel velocity and pressures as *b* but 25 p.p.m. polyacrylamide solution instead of pure water.  $Re = 2 \times 10^5$ ,  $\sigma = 0.5$ .

The demonstration in polymer solution flow of a destabilising effect on transition, together with the known diminishing of turbulence production at higher Reynolds numbers should have an important influence on the development of theoretical explanations of the mechanism of polymer drag reduction.

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1. Giles, W. B. & Pettit, W. T. *Nature* **216**, 470 (1967).
2. Castro, W. & Squire, W. *Appl. Sci. Res.* **18**, 81 (1967).
3. White, W. D. & McEligot, D. M. *Trans ASME, J. Bas. Engng.* **92D**, 411 (1970).
4. Little, R. C. *et al. J. & EC Fundamentals*, **14**, 283 (1975).
5. Zakin, J. L., Ni, C. C., Hansen, R. J. & Reishman, M. M. *Proc. IUTAM Symp. Structure of Turbulence and Drag Reduction* Washington (1976).
6. Brennen, C. *J. Fluid Mech.* **44**, 33 (1970).
7. Hoyt, J. W., Taylor, J. J., & Runge, C. D., *J. Fluid Mech.* **63**, 635 (1974).
8. Hoyt, J. W. & Fabula, A. G. *Proc 5th Symp. Naval Hydrodynamics* 947 (1964).

## Time sharing and body partitioning in bat-plant pollination systems

IN the dry months from January to March in Central America there is an abundance of flowering trees and shrubs<sup>1-3</sup> and many of them are pollinated by bats. When collecting nectar, the bats visit various species of plants<sup>4-6</sup>, and in periods of peak abundance of flowers they make increasingly frequent visits. With flowering apparently synchronised<sup>3,4</sup>, there seems to be a good chance that flowers may not always receive conspecific pollen, thus reducing their seed set and fitness. The study reported here suggests that this chance is minimised because nectar and pollen are available at different times on different flower species, and because some species deposit and receive pollen at distinct regions of the bats' bodies.

Interspecific competition among plants sharing common elements of the pollinator fauna is seen in entomophilous systems when weeds or native plants prove superior to crop plants<sup>7,8</sup>. The disadvantage of being a minority species in such a community free-for-all has been shown by Levin and Anderson<sup>9</sup>—given a finite number of visits by pollinators, sharing of pollinators by plant species might decrease the chances of appropriate pollination for less numerous species, resulting in lowered seed set and eventual elimination from the community. Thus, a hypothetical case can be made for mixed species associations becoming increasingly oligotypic. But real tropical communities persist in polytypy, which shows that plants avoid this dilemma.

Sympatric insect-pollinated plants with similar phenologies seldom interbreed because they: (1) are incompatible; (2) 'use' relatively specific pollinators; (3) deposit pollen on different parts of the pollinator's body, and (4) present pollen and/or nectar rewards at different times<sup>10,11</sup>. I suggest that time sharing and body partitioning are 'escapes' from competition available to some bat-adapted plants.

The feeding habits of bats have been investigated previously by stomach and faecal analysis<sup>5,6,12</sup>, and by swabbing the fur with an adhesive substance and then examining the swabbed material microscopically<sup>4,13</sup>. Workers using such pooled pollen analyses have interpreted mixed pollen loads to indicate lack of discrimination by the bats. For example, Heithaus *et al.*<sup>4</sup> found six species of pollen in 42% of 527 loads examined and concluded that: "The high proportion of mixed pollen loads ... suggests low flower constancy in bats". But the methods used in all these studies did not or could not take into account the distribution of pollen temporally or topographically. For plants to have the benefits of pollinator specificity, bats need not feed only on one species. Effectiveness of pollination would be enhanced if bats confined each of their several nightly visits for feeding to a single species, or if they visited different species on different nights or at different times.

While working in Costa Rica and British Honduras in March 1974, 1975 and 1976, I netted bats in several sites between 1930



**Table 1** Pollen grains from fur of *Glossophaga soricina*, March 1974 and 1975

Central farm, British Honduras <i>N</i> = 26							
Time	<i>Bauhinia</i> (large-small)		<i>Inga</i>	<i>Roupala</i>	<i>Ipomoea</i>	<i>Calliandra</i>	Bombacaceae
1900-2100			1,600	200			
2200-0200					557	10	
0200-0400	1,267	110	12		1		123
0400-0600		200	2	1			
Santa Rosa, Costa Rica <i>N</i> = 22							
1900-2100			500			220	
2000-0200							913
0200-0400	870	20	3				640
0400-0800	300	121	17				2

and 0530 h. I swabbed pollen from the heads, shoulders, venters and rumps of glossophagine bats, making separate slide preparations for each anatomical region and noting the times of capture. Table 1 shows that *Glossophaga soricina* Pallas concentrates on *Inga* (Mimosaceae) early in the evening and switches to *Bauhinia* (Caesalpinaceae) towards dawn.

The way in which plants may reinforce or entrain such behaviour is shown in Fig. 1; nightly nectar flow is timed correspondingly. *Inga* anthers are ripe between 2000 and 2100 h, whereas *Bauhinia* anthers do not dehisce until the early morning (0400-0600 h). This suggests that the availability of pollen is also co-adapted with bat schedules. The visual acuity of nectar bats is fairly good<sup>14</sup>, and the well developed olfactory lobes and vomeronasal organ<sup>15-17</sup> indicate that olfaction plays a large role in their orientation. Flower discrimination and concomitant time-entrainment should be no more difficult for bats than for bees. As Darwin pointed out<sup>18</sup>, such constancy affords the pollinator more efficient foraging as well as facilitating appropriate pollination. Further bat and plant studies in other months or in different sites may reveal more of this trend to time partitioning.

While netting along a transect from Monte Verde, Guanacaste, Costa Rica to a point 5 km west, I captured eight female and four male *Anoura geoffroyi* Grey. Two yielded pure *Mucuna* (Papilionaceae) pollen, probably *M. urens*, placed ventrally. The other 10 carried mixed loads, *Mucuna* on the venter, *Crescentia* (Bignoniaceae) on the shoulders and mid-dorsum and pure *Inga* (six bats) or mixed *Inga* and unidentified Bombacaceae (four bats) on the face and neck. Essentially the plants were using three different and fairly constant pollinators.

These data do not preclude overlap in pollinator 'use' by many plants. For example, Alvarez and Gonzalez Quintero<sup>5</sup> found mixed pollen loads on *Glossophaga* and *Anoura*; four or five genera were often well represented. Although the methods used (stomach and faecal analysis) may have hidden a microtemporal

structure which could be revealed by further work, it is unlikely that all genera found in pooled pollen samples were involved in time sharing.

Another example is provided by *Bauhinia unguolata*, *Bombacopsis quinata*, *Ceiba pentandra*, *Luchea speciosa* and *Pseudobombax septenatum*, which can bloom synchronously in the same site<sup>19</sup>. All these bat-adapted species have morphological adaptations to deposit pollen on the head, suggesting that floral homologues 'use' identical pollinators. As Baker<sup>20</sup> indicated, taxonomically unrelated flowers may well be able to share a pollinator where the supply of that animal is adequate. Only when pollinators are scarce would such behaviour on the part of the plants become disadvantageous. At such times sympatric species which partition pollinators may be able to co-exist more successfully than those that do not<sup>21</sup>. Competition resulting from a low density of pollinators would then reinforce floral differences or promote such differentiation, reinforcing and maintaining community polytypy.

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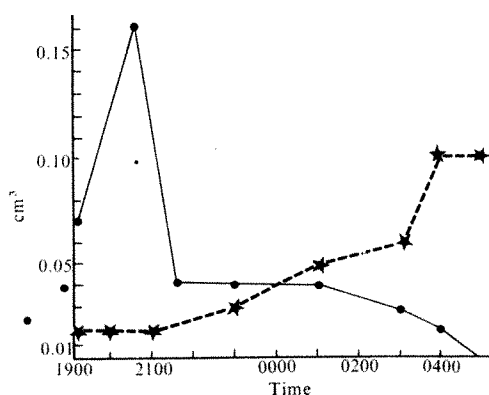
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1. Janzen, D. *Evolution* **21**, 620-637 (1967).
2. Croat, T. *Ann. Miss. Bot. Gdn* **56**, 295-307 (1969).
3. Frankie, G., Baker, H. B. & Opler, P. *J. Ecol.* **62**, 881-919 (1974).
4. Heithaus, R., Fleming, T. & Opler, P. *Ecology* **56**, 841-859 (1975).
5. Alvarez, T. & Gonzalez Quintero, L. *An. Esc. Nac. Cienc. Biol.* **18**, 1-77 (1970).
6. Howell, D. *Comp. Biochem. Physiol.* **48**, 263-276 (1974).
7. Friden, F., Eklund, L. & Bingle, S. in *Proceedings of the First International Symposium on Pollination* 17-26 (Copenhagen, 1962).
8. Holm, S. *Ann. Rev. Entomol.* **11**, 155-182 (1966).
9. Levin, D. & Anderson, W. *Ann. Nat.* **104**, 455-467 (1970).
10. Free, J. in *Reproductive Biology and Taxonomy of Vascular Plants* (ed. Hawkes, J. G.) 76-91 (Pergamon, New York, 1966).
11. Percival, M. *New Phytol.* **49**, 40-63 (1950).
12. Goodwin, G. G. & Greenhall, A. *Bull. Am. Mus. Nat. Hist.* **122**, 187-302 (1961).
13. Howell, D. & Burch, D. *Rev. Biol. Trop.* **21**, 281-294 (1974).
14. Suthers, R. in *Biology of Bats* (ed. Wimsatt, W.) 265-310 (Academic, New York, 1970).
15. Mann, G. *J. comp. Neurol.* **116**, 135-144 (1961); *Invest. Zool. Chilenas* **9**, 1-93 (1963).
16. Bhatnagar, K. & Kallen, F. *J. Morph.* **142**, 71-90 (1974); *Am. J. Anat.* **139**, 167-190 (1974).
17. Cooper, J. & Bhatnagar, K. *J. Anat.* **122**, 571-601 (1976).
18. Darwin, C. *The Effects of Cross and Self Fertilization in the Vegetable Kingdom* (Murray, London, 1876).
19. Holdridge, L., Grenke, W. C., Hatheway, W. H., Liang, T. & Tosi, J. Jr *Forest Environments in Tropical Life Zones* (Pergamon, New York, 1971).
20. Baker, H. *Science* **139**, 877-883 (1963).
21. Grant, V. *Evolution* **3**, 82-97 (1949).

**Fig. 1** Nightly nectar flow in two bat-pollinated species. Nectar was extracted with a 1-cm<sup>3</sup> syringe and blunt needle. Points for each species represent averages of 10 flowers from two trees. ●—●, *Inga marginata*; — — —, *Bauhinia unguolata*.



## Possible case of aggressive mimicry in a neotropical scale-eating fish

*Probolodus heterostomus*, one of the scale-eating characid fishes, is found in the coastal rivers of southeastern Brazil from Espírito Santo to São Paulo<sup>1-3</sup>. The species is usually placed in the subfamily Cheirodontinae<sup>2-4</sup> although a taxonomic affinity with the Tetragonopterinae has been suggested<sup>1</sup>. The latter view was apparently based largely on the morphological similarity of *Probolodus* with some species of *Astyanax* and especially *A. fasciatus*, a member of the

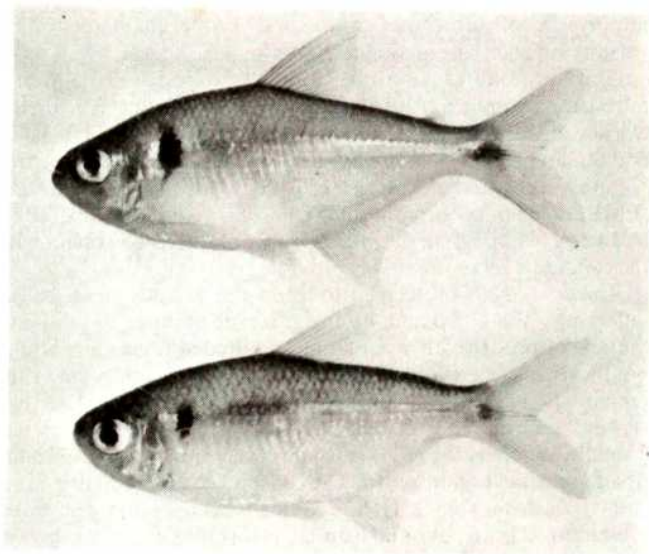


Fig. 1 *P. heterostomus* (upper) and *A. fasciatus* (lower) from the Rio Paraibuna, São Paulo. The colour of both fishes is also very similar; actual size of *Probolodus* 76 mm.

Tetragonopterinae. The external similarity between the two species, which are sympatric over the Rio Paraíba drainage, is indeed striking (Fig. 1). But data accumulated from population samples, stomach contents and the behaviour of individuals maintained in aquaria suggest that *Astyanax* is the principal prey of *Probolodus*, at least in certain areas of its range. I suggest here that the similarity between these two genera is a form of aggressive mimicry<sup>3,6</sup> irrespective of taxonomic affinities.

Thirty-five individuals of *P. heterostomus* (54–104 mm) were caught in the Rio Paraibuna, São Paulo (22°23'S, 45°40'W), on five occasions between May and July 1977. The fish were collected by means of casting net, bow-net and hook. On the same occasions a general collection of other fishes was also made. On all occasions *Probolodus* was caught together with *A. fasciatus* (total 35 individuals compared with 409) which strongly suggests that these two species shoal together or at least live in close proximity. On three occasions, 24 individuals of the curimatid *Curimatus gilberti* were also caught together with *Probolodus* and *Astyanax*. Other scale-bearing fishes found sympatrically with *Probolodus* were the erythrinid *Hoplias* (one species); the characids *Astyanax* (two additional species), *Hyphessobrycon* (one species) and *Oligosarcus* (one species); the anostomid *Leporinus* (two species), and the cichlids *Geophagus* (one species), *Cichla* (one species) and the introduced *Tilapia* (one species). For these, no comparable numerical data were obtained but they were far less numerous than *A. fasciatus* and only incidentally collected together with it and *Probolodus*.

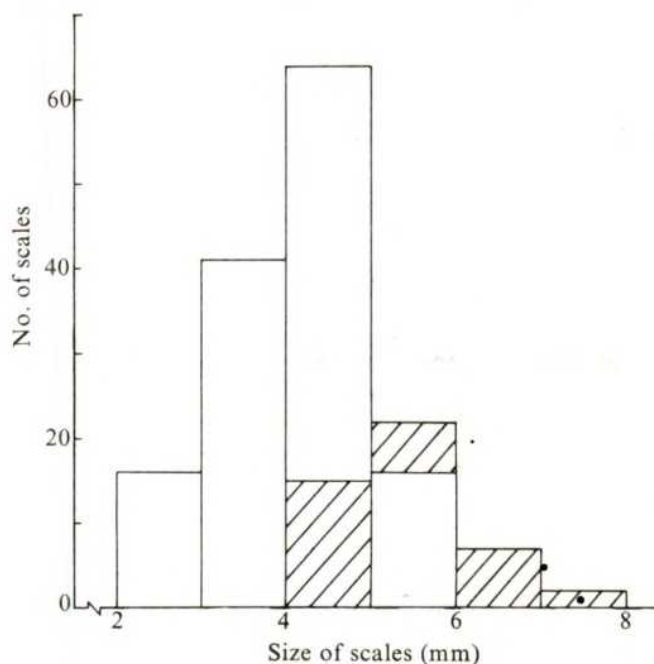
With the exception of four individuals which were maintained alive all *Probolodus* were killed immediately and preserved. These preserved specimens were scored for length and their stomach contents were analysed. Relatively undigested scales from 31 stomachs were stained with alizarin red and compared with a reference collection of scales from the fishes of the general collection (Fig. 2). In this sample 183 scales were clearly recognised as from *Astyanax* and *Curimatus*. It was not possible to identify with certainty the remaining, more digested scales, but only eight were of the ctenoid type (attributable to *Tilapia*) while all others, about 600, were cycloid of the *Astyanax* and *Curimatus* type. Other food items included plant material and insects.

The four live fish (64–82 mm) were maintained separately in aquaria (50–160 l) and used in observations of feeding behaviour. In one series of observations two to four individuals of *Astyanax* (36–115 mm) were placed together with

each *Probolodus*, and the groups were observed in sessions of 15–40 min for about 40 h. Soon after the introduction of *Astyanax*, *Probolodus* shoaled with them, and at intervals fed on their scales. In the most frequently observed feeding strategy *Probolodus* followed closely an individual of *Astyanax*, manoeuvred itself obliquely to the rear of the victim and struck at its flanks, securing one to a few scales on each successful attack. The scales seemed to be removed merely by a gaping and closing of the mouth during the strike, sometimes accompanied by a quick lateral movement of the head. The scales were swallowed immediately and *Probolodus* resumed shoaling. During the first day or so *Astyanax* shoaled together with *Probolodus* and apparently failed to recognise it as a 'predator'. Subsequently, *Astyanax* avoided close proximity with *Probolodus* and, to a certain extent, with each other. This latter behaviour is perhaps the result of repeated attacks on the same individuals in the confinement of the aquarium. In natural conditions the possibility of recognising *Probolodus* as a predator would be diminished because attacks would be 'diluted' over a greater number of victims. Fish less than half the size of *Probolodus* individuals, or more than one and a half times as large, were unmolested, which suggests that prey of more or less the same size as the predator are preferred.

In other observations, two individuals of diverse species were introduced separately into the aquarium with a *Probolodus* individual. These species included the cichlid *Geophagus brasiliensis* (60–81 mm), the characid *Bryconamericus* sp. (50–55 mm), the cyprinid *Carassius auratus* (41–53 mm) and the poeciliid *Phalloceros caudimaculatus* (22–32 mm). After initial attacks both *Bryconamericus* and *Carassius* avoided the close presence of *Probolodus*, hid and needed to be pursued. Only the largest *Probolodus* (82 mm) attacked *Geophagus*, and to a much lesser extent than the other species, while the small *Phalloceros* went unmolested. The latter observation confirms the size preference shown previously by *Probolodus*. In a final series of observations, both *Astyanax* (two individuals) and *Geophagus* (one individual) were introduced into an aquarium with *Probolodus*.

Fig. 2 Number and size of 183 relatively undigested scales from stomach analyses of 31 individuals of *P. heterostomus*. Size extremes for each genus may be found in the same individual prey and also in the stomach of the same predator. Open columns, *Astyanax*; hatched columns, *Curimatus*.





After 24 h *Astyanax* had suffered much greater scale loss than *Geophagus*.

Taken together, the accumulated data provide support for the hypothesis of aggressive mimicry<sup>3,6</sup> in which *Astyanax* is the prey-model and *Probolodus* is the predator-mimic. First, *P. heterostomus* and *A. fasciatus* have a striking overall similarity to each other. Aquarium observations indicate that *Astyanax* tolerated *Probolodus* and failed to recognise it as a potential predator, at least until repeated scale-biting in the conditions of a small aquarium caused a break-down of the shoaling habit. Second, both species are sympatric in the Rio Paraibuna (and over the Rio Paraíba drainage) and apparently live in mixed shoals or in close proximity. The proportion of *Probolodus* individuals to *Astyanax* in these areas seems to be roughly 1:10 which again fits the mimicry requirements. Third, *Probolodus* is a specialised scale-eater with a preference for prey more or less its own size and which is closely followed and struck from the rear. Stomach analyses of individuals caught in the Rio Paraibuna show preponderance of *Astyanax* scales.

A comparable case of mimicry has been reported for the African scale-eating cichlid fish *Corematodus shiranus* which closely resembles and shoals with the cichlid species *Tilapia squamipinnis*<sup>7,8</sup>. In such cases, the mimicry of the predator apparently facilitates its predatory success by allowing it to maintain close proximity with its potential prey<sup>3-9</sup>. This is the classic tactic of the wolf in sheep's clothing. Even if *Probolodus* is not so selective as it seems from my data it may still be advantageous to have the appearance of an ubiquitous and harmless fish, to facilitate the approach to an intended victim. A comparable strategy is used by a bird of prey<sup>10</sup> and some marine fishes<sup>11,12</sup>.

As *A. fasciatus* is not found over the entire range of *Probolodus*, it is probable that in other major river drainages this latter fish associates with other similar species of *Astyanax* (in the Rio Ribeira do Iguape, *A. ribeirae* is a likely candidate) or even such tetragonopterines as *Deuterodon iguape* which also has the general appearance of *Astyanax*.

Drs P. E. Gibbs, E. O. Willis and P. Montouchet reviewed the manuscript and Dr H. E. Britski confirmed the identity of the fishes.

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1. Roberts, T. R. *Proc. Calif. Acad. Sci.* **38**, 383-390 (1970).
2. Eigenmann, C. H. *Memoirs Carnegie Mus.* **7**, 1-99 (1915).
3. Fowler, H. W. *Arq. Zool. S. Paulo* **6**, 1-204 (1948).
4. Britski, H. A. in *Poluicao e Piscicultura* 79-108 (CIBPU & Fac. Saúde Pública USP, São Paulo, 1973).
5. Wickler, W. *Mimicry in Plants and Animals* (McGraw Hill, New York, 1968).
6. Edmunds, M. *Defence in Animals* (Longman, Harlow, 1974).
7. Trewavas, E. *Nature* **160**, 120 (1947).
8. Fryer, G. & Iles, T. D. *The Cichlid Fishes of the Great Lakes of Africa* (Oliver & Boyd, Edinburgh, 1972).
9. Curio, E. *The Ethology of Predation* (Springer, Berlin, 1976).
10. Willis, E. O. *The Condor* **65**, 313-317 (1963).
11. Losey, G. S. *Pacif. Sci.* **26**, 129-139 (1972).
12. Springer, V. G. & Smith-Vaniz, W. F. *Smithson. Contr. Zool.* **112**, 1-36 (1972).

## A new insect chemosterilant isolated from *Acorus calamus* L.

PLANT extracts have been tested for insecticide and juvenile hormone<sup>1</sup> activity and some have been found with anti-juvenile hormone activity<sup>2</sup>. We have suggested<sup>3,4</sup> that an essential oil of *Acorus calamus* L. inhibits interstitial cell activity; this would represent a new concept in insect chemosterilisation. We report here the identification of the component of the oil that is responsible for that inhibition; we have also established the

importance of substitute groups and a side chain by testing various allyl benzene analogues.

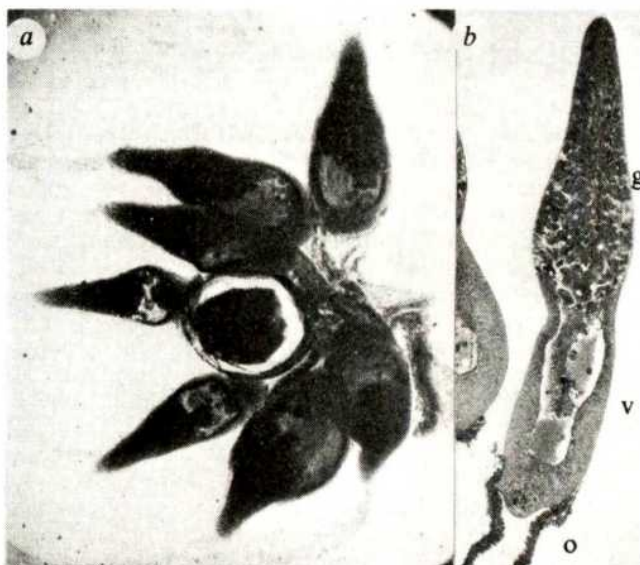
Using procedures described before<sup>3,4</sup>, we tested 3rd, 4th and 5th instar nymphs and the adults of *Dysdercus koenigii* for the efficacy of the inhibitory agent. Victoria blue<sup>5</sup>, aldehyde fuchsin<sup>6</sup> and Sudan black B were used to test for the appearance of neuro-secretory cells and interstitial cells (IC). Farnsyl methyl ether (FME), a juvenile hormone analogue, was given topically (10 µg and 12 µg per insect) to the insects before and after treatment with the candidate oil to reveal any physiological reversal.

*Acorus calamus* oil seemed to affect the various larval stages differently. While 3rd and 4th instar larvae moulted normally to the next instars, the 5th instar larvae moulted normally into adults but the ovaries were irreversibly affected. In such adults the ovary remains permanently immature (Fig. 1a). Longitudinal section through such an ovary revealed a well developed tropharium but a small vitellar region (Fig. 1b), composed only of interfollicular tissue, and no organised follicles. Isolation of the active principle<sup>7</sup>, β-asarone (Fig. 2, I) showed that it brought about a similar inhibition. The compound caused resorption in many gravid insects leading ultimately to complete regression of the ovaries. To find which of the substituent groups in the aromatic ring or the side chain of β-asarone was specifically inhibitory, trials were done with different allyl benzene compounds (Fig. 2). A wide spectrum of activity was observed (Table 1).

Table 1 shows that compound I severely depressed the development of the gonads. The neuro-secretory cells (NSC) revealed by both Victoria blue and aldehyde fuchsin were scored according to Schooneveld<sup>8</sup> and Johnson *et al.*<sup>9</sup>. The studies revealed no inhibition in the NSC's and the density of granules was nearly equal to that in controls. In cases where higher dosages (above 30 µl per 1,500 cm<sup>3</sup> of air space) were tried, however, a mild inhibition in the secretion from NSC was observed. There is no reason to suppose that NSC activity is involved in the chemosterilant action of β-asarone, because it has been reported that extirpation of the NSC within 30 h of the moult to adult does not affect subsequent vitellogenesis in *D. cingulatus*<sup>10</sup>.

Because the treated insects showed a normal moulting pattern we conclude that the oil does not adversely affect ecdysone release. As previously shown<sup>11</sup>, on treatment with higher doses, the moulting insect becomes lethally entangled in its own half cast moult. This may be because the larger dose of oil inhibits the release of secretion by NSC, which then delays the release of ecdysone and thus seriously impedes ecdysis.

**Fig. 1** a, Ovary of 6-d-old adult treated with *Acorus* oil. Note the well developed germarium and oocytes as spherical bodies in vitellar cavity. × 15. b, Longitudinal section through the ovariole of above ovary. Note the well developed tropharium (g) a short vitellarium (v) and oviduct (o) × 75.





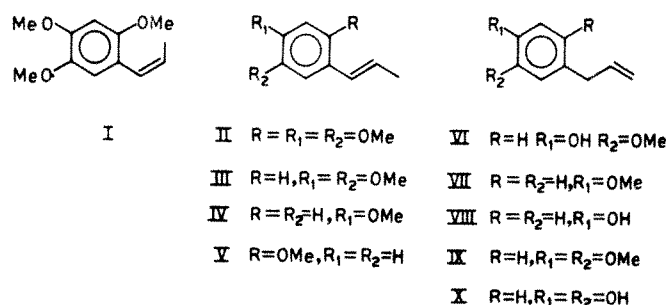


Fig. 2 Structural formulae of compounds used.

The anti-juvenile hormones precocene I and II possess a significant measure of structural similarity with  $\beta$ -asarone, and both types of compound cause sterility in adult female insects. But they probably have different modes of action. Thus precocenes are believed to act by blocking the synthesis and release of juvenile hormones, which leads to the formation of precocious adults when the larvae of sensitive species are treated<sup>2</sup>. By contrast,  $\beta$ -asarone did not cause precocious metamorphosis in *D. koenigii* in our experiments. Furthermore, we observed no physiological reversal of the  $\beta$ -asarone effect on insect gonads when FME was applied topically to the insects before or after  $\beta$ -asarone treatment. These observations were made on adults up to 7d after moulting, which coincides with the first oviposition of control insects.

The molecular structure of the effective compound from oil of *A. calamus* is given in Fig. 2 I. The position of different groups in the aromatic ring is known to play an important part in various activities, as shown by some allelochemicals<sup>1,2</sup>, where the position of the hydroxyl groups is responsible for activities such as anti-assimilation and reduction of survival or pupation. Similarly in our study the position of the *O*-methyl groups on the ring, and of the double bond in the side-chain of  $\beta$ -asarone, plays an important part in the antigonadal activity (Table 1, Fig. 2). After testing the various compounds it was found that the *O*-methyl group at position R in the ring (Fig. 2, V) is important for the resorption of the oocytes, which is further supported by the suppression in the ovary due to the *O*-methyl group at R<sub>2</sub> (as in Eugenol, VI and methyl isoeugenol, III—Fig. 2). The suppression in the ovary due to the *O*-methyl group at R<sub>2</sub> seems true of precocene II<sup>2</sup> also because the removal of the *O*-methyl group from R<sub>2</sub> position in that molecule also reduces activity (precocene I of Bowers<sup>2</sup>). Comparison of the activities of compounds I and II (Table 1) suggests that the *cis* configuration of the side-chain double bond in  $\beta$ -asarone is important for antigonadal activity. It is interesting, however, that III which has a *trans* configuration, but lacks the *O*-methyl group at position R also has significant suppression activity.

Table 1 Activities of test compounds

Compounds	Activity		
	10 $\mu$ l per 1,500 cm <sup>3</sup> air space	20 $\mu$ l per 1,500 cm <sup>3</sup> air space	30 $\mu$ l per 1,500 cm <sup>3</sup> air space
I	Antigonadal	Antigonadal	Antigonadal
II	Nil	Nil	Nil
III	Nil	Mild yolk deposition*	Antissimulant + no yolk deposition
IV	Nil	Toxic	Toxic
V	Nil	Nil	Antigonadal
VI	Nil	Antissimulant + no yolk deposition	Toxic
VII	Nil	Nil	Nil
VIII	Nil	Nil	Nil
IX	Antifeedant	Toxic	Toxic
X	Nil	Nil	Nil

An antigonadal compound sterilised either sex, agglutinated sperm and caused resorption from terminal oocytes to apex of vitellarium. In antissimilants the alimentary canal was full and the fat body highly reduced and in antifeedants the alimentary canal was always empty.

\*Mild yolk deposition: the effect was the same in all oocytes.

We conclude that  $\beta$ -asarone is neither a juvenile hormone, nor an anti-allatotrophic compound (precocene I and II of Bowers<sup>2</sup>), and with its specific antigonadal functions, it may represent a new type of antigonadal agent which may afford a new and safe approach towards insect control.

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- Jacobson, M., Redfern, R. E. & Mills G. D., Jr *Lloydia* **38**, 455–474 (1975).
- Bowers, W. S., Ohta, T., Cleere, J. S. & Marsella, P. A. *Science* **193**, 542–547 (1976).
- Saxena, B. P., Koul, O. & Tikku, K. *Acta ent. Bohemosl.* **74** (in the press).
- Saxena, B. P. & Mathur, A. C. *Experientia* **32**, 315–316 (1976).
- Dogra, G. S. & Tandan, B. K. *Q. J. Microsc. Sci.* **105**, 455–466 (1964).
- Humanson, G. L. *Animal Tissue Techniques* 332–333 (Freeman, San Francisco, 1967).
- Jacobson, M., Keiser, I., Miyashita, D. H. & Harris, E. J. *Lloydia* **39**, 412–415 (1976).
- Schooneveld, H. *Neth. J. Zool.* **20**, 151–237 (1970).
- Johnson, E., Saum, T., McDaniel, C. N. & Berry S. J. *J. Insect Physiol.* **22**, 713–723 (1976).
- Jalaja, M. & Prabhu, V. K. *Entomol.* **2**, 17–29 (1977).
- Saxena, B. P. & Srivastava, J. B. *Ind. J. exp. Biol.* **10**, 391–393 (1972).
- Reese, J. C. & Beck, S. S. *Ann. ent. Soc. Am.* **69**, 999–1003 (1976).

## Assignment of aberration breakpoints in banded chromosomes

CHROMOSOME banding techniques<sup>1–4</sup> have made possible not only the detection but also a more accurate characterisation of the structural chromosomal changes encountered in clinical and experimental work. Using internationally agreed band patterns and nomenclature<sup>5</sup>, it is customary to assign breakpoints to aberrations such as translocations on the assumption that these 'breakpoints' correspond to the positions of breakage and rejoining which gave rise to the configuration observed. This assumption is frequently invalid, because it does not seem to be generally realised that the system carries (at least for chromosome-type exchanges) an inherent three-band uncertainty. This means that the most obvious breakpoint is not necessarily the site of transfer or rearrangement of the genetic material.

The assignments of 'breakpoints' is essentially an exercise in pattern recognition. Presented with a linear array of alternating dark and light segments against a light background (for example, a typical G-banded chromosome set viewed with conventional light microscopy), the human eye automatically concentrates on the disposition of the dark bands. We are much more conscious of differences in size, spacing and staining intensity of dark bands than of light bands although such differences occur in the latter.

Interruptions or breaks in an expected sequence are therefore interpreted in terms of the dark band pattern, and the pattern disruption point (PDP) will be assigned to a light band. Figure 1 illustrates this for a 6:10 human reciprocal translocation using the Paris Conference (1971) diagrams and nomenclature<sup>5</sup>. The left-hand pairs represent the G band patterns of the normal and derivative chromosomes. Obvious pattern disruption occurs at 6q21 and 10q22, that is a light/light-band exchange. But, essentially the same pattern (and derivative chromosome lengths and arm ratios) could be achieved by dark/dark-band exchanges on either side of this light-band, that is 6q16/10q21 or 6q22/10q23, but because of the eye's preoccupation with dark-bands, this is not immediately obvious.

This three-band uncertainty becomes clear when the same translocation is R banded<sup>6</sup>, a technique whereby the Giemsa staining of the bands is reversed in a more or less reciprocal manner (Fig. 1, right-hand pairs). The eye transfers attention to the new

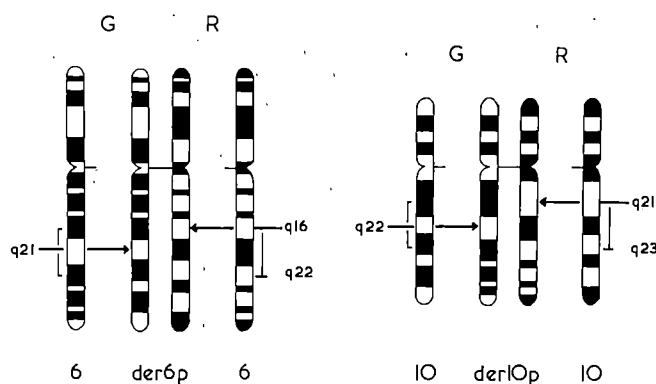


Fig. 1 The derivative chromosomes of a human 6:10 reciprocal translocation compared with normal homologues. Left-hand pairs, G-band pattern; right-hand pairs, R-band pattern. Arrows indicate the pattern disruption points (PDP), and the three-band uncertainty zone is bracketed.

dark-band sequence so that the PDP again falls in a light band. This corresponds to one or other of the dark G bands which make up the trio of uncertainty. The breakpoint seems to have jumped a band, but only as the result of an optical artefact; it is not necessary to infer that exchange at a band junction is involved<sup>7</sup>. In the example of Fig. 1, the choice of PDP depends on the assessment of the R dark bands 6q21 and 10q22. Relative size differences are obvious in the diagram, and careful measurement will probably implicate 6q21 and 10q22 as the most likely breakpoint positions. In the real cell, such differences are seldom obvious, as we are working near the limits of optical resolution, and the quality of the banding is rarely uniform throughout the chromosome complement, so that homologue differences are often present.

In clinical work, where a particular translocation can be examined by different techniques and in several cells, subtle differences in size, staining intensity and conformation, impossible to depict in a diagram, may allow assignment of the breakpoint with reasonable certainty, but not in every case. In experimental work, particularly with primary chromosome-type changes, usually only one example is available, and the three-band uncertainty frequently remains. Where uncertainty exists it may be safer to use a more non-committal term like PDP.

It is often said that most chromosome-type exchange breakpoints occur in G light-bands (or weak fluorescing bands which are mostly identical to light bands)<sup>8-11</sup>. This could be largely an artefact of pattern-recognition.

Non-exchange aberrations such as simple terminal deletions do not, however, have the three-band uncertainty. This is also the case for most chromatid-type aberrations, because sister-chromatid pairing affinity ensures a banded 'control' chromatid in close proximity to the exchange point. Thus band-for-band comparisons can be made<sup>12-14</sup>. When this is done for X-ray-induced exchanges there is a clear indication of more or less exclusive light/light band involvement.

Free participation of both dark and light bands should lead to ~ 50% dark/light-band exchanges, which would be recognised by the creation of an extra dark band in one of the derivative chromosomes. Such cases seem to be very rare, implying that either exchanges are predominantly light/light or dark/dark or that partial bands are unstable and tend to merge with bands adjacent to the point of exchange. A similar argument applies to exchanges involving band junctions, interfaces<sup>15</sup>, or 'interbands'<sup>7</sup>. A high frequency of free interface/interface exchange would produce frequent band fusion in one derivative and concomitant band loss in the other. Such events are seldom seen.

Terms like band 'interface' and 'interband' should be used with caution. Sharp dark/light band junctions only exist on diagrams. The metaphase chromatid is a coiled<sup>16</sup> or double-coiled<sup>17</sup> structure, and there have been several suggestions that bands and coils are related<sup>18-20</sup>. Thus a transverse line across a chromatid will

run more or less parallel to the pitch angle of the coil, and is therefore unlikely to represent a sharp boundary for breakage purposes.

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1. Caspersson, T., Lomakka, G. & Zech, L. *Hereditas* **67**, 89 (1971).
2. Sumner, A. T., Evans, H. J. & Buckland, R. A. *Nature new Biol.* **232**, 31 (1971).
3. Seabright, M. *Lancet* **ii**, 971 (1971).
4. Schnedl, W. in *Chromosome Identification* (eds Caspersson, T. & Zech, L. **34** (Academic, New York, 1973).
5. Paris Conference (1971). *Standardization in human cytogenetics. Birth Defects: original article series* **8**, 7 (National Foundation March of Dimes, 1972).
6. Dutrillaux, B. & Lejeune, J. *C.R. hebdo. Séanc. Acad. Sci., Paris* **272**, 2638 (1971).
7. Dutrillaux, B., Cauterier, J., Vlegas-Péguignot, E. & Schaison, G. *Hum. Genet.* **37**, 65 (1977).
8. Seabright, M. *Chromosoma* **40**, 333 (1973).
9. Bigger, T. R. L., Savage, J. R. K. & Watson, G. E. *Chromosoma* **39**, 297 (1972).
10. Holmberg, M. & Jonasson, J. *Hereditas* **74**, 57 (1973).
11. San Roman C. & Bobrow, M. *Mutat. Res.* **18**, 325 (1973).
12. Savage, J. R. K., Watson, G. E. & Bigger, T. R. L. in *Chromosomes Today 4* (eds Wahrman, J. & Lewis, K. R.) **267** (Wiley, New York, 1973).
13. Savage, J. R. K. *J. med. Genet.* **14** (in the press).
14. Savage, J. R. K., Bigger, T. R. L. & Watson, G. E. in *Chromosomes Today 5* (eds Pearson, P. L. & Lewis, K. R.) **281** (Wiley New York, 1973).
15. Buckton, K. E. *Int. J. Radiat. Biol.* **29**, 475 (1976).
16. Ohnuki, Y. *Chromosoma* **25**, 402 (1968).
17. Iino, A. *Cytogenetics* **10**, 286 (1971).
18. Kato, H. & Yosida, T. H. *Chromosoma* **36**, 272, (1972).
19. Bigger, T. R. L. & Savage, J. R. K. *Cytogenet. Cell Genet.* **15**, 112, (1975).
20. Takayama, S. *Chromosoma* **56**, 47, (1976).

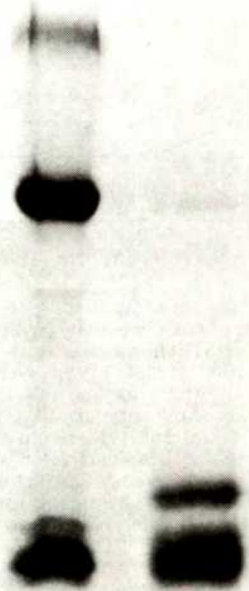
## *Tetrahymena pyriformis* recovers from antibody immobilisation by producing univalent antibody fragments

MANY protozoa possess mechanisms by which they are able to recover from immobilisation by specific antibodies. Paramecia have been shown to shed their ciliar antigens in response to specific divalent antibodies. After shedding the antibody bound antigens the animals present new antigens of different specificity<sup>1</sup>. It has been suggested that this modulation process also has a role in antigen variation in trypanosomes<sup>2</sup>. While modulation of serotypes in *Tetrahymena pyriformis* is primarily provoked by temperature shifts or changes in culture conditions<sup>3</sup>, induction of modulation in response to antibody agglutination has been reported<sup>4,5</sup>. *Tetrahymena* recovery from antibody provoked immobilisation has also been reported to occur by a nonspecific process not involving a change in serotype. Immobilised animals have been shown to secrete a substance which permits their recovery and protects animals of unrelated serotype from homologous anti-serum<sup>6-8</sup>. We have further examined the way that *Tetrahymena* recover from antibody provoked immobilisation. This process, which we call fabulation, apparently involves the proteolytic cleavage of both bound and free immunoglobulin to produce univalent fragments which bind or remain bound to the ciliar antigens and protect the animals from further antibody treatment with no apparent effects on cell growth. The cleavage seems to be catalysed by both cell bound and secreted proteases.

When *Tetrahymena* of syngen 9 are treated with specific antisera they are rapidly immobilised and often agglutinated. Rabbit antisera to *T. pyriformis* were prepared either by the method described in ref. 4 or by two intradermal injections of  $5 \times 10^5$  animals in complete Freund's adjuvant at 6-week intervals, followed by two intravenous injections of  $10^4$  sonicated animals at 2-week intervals. Rabbits were bled 6 d after the last injection. Immobilisation and agglutination tests were performed as described in ref. 4, except that the cell concentration was 2–4,000 per ml.

We have observed that when immobilised animals are left at room temperature for 1–2 h in the presence of antiserum all the animals are found to have recovered and swim freely. Further treatment of the recovered animals with antiserum of the same specificity has no effect. Also, addition of antiserum directed to other serotypes which the animals are capable of expressing has no effect. The possibility that the recovered animals had modulated their serotype and presented a hitherto unknown antigen was tested by injecting the recovered animals into rabbits. We found, however,





**Fig. 1** Effect of *Tetrahymena* cells on  $^{14}\text{C}$ -labelled mouse immunoglobulin.  $^{14}\text{C}$ -labelled mouse immunoglobulin, prepared by incubating spleen cells from trypanosome infected mice in a mixture of  $^{14}\text{C}$  amino acids, was a gift from Dr B. Bernstein.  $10^4$  *Tetrahymena* grown as described in ref. 6 were incubated with  $5\ \mu\text{l}$  of  $^{14}\text{C}$ -immunoglobulin (12,000 c.p.m.) in  $10^{-2}\text{M}$  phosphate buffer pH 7.5 containing  $10^{-3}\text{M}$  EDTA and  $10^{-3}\text{M}$  dithiothreitol. After 1 h at  $37^\circ\text{C}$ , 2 volumes of  $2\times$  sample $^{12}$  buffer were added and the samples were boiled for 2 min. They were then analysed by electrophoresis on 10% polyacrylamide gels as described by Laemmli $^{13}$ . The gels were dried and autoradiographed. The left lane represents the immunoglobulin preparation incubated without *Tetrahymena* cells. The right lane represents the preparation incubated with the cells.

that this resulted only in the production of very weak sera against the original serotype. It thus seems unlikely that the animals recover by a process of modulation of their ciliar antigens. If the recovered animals are cultivated in antiserum free medium (containing normal rabbit serum in the place of anti-*Tetrahymena* rabbit serum) they become sensitive to the original antiserum within 2–3 generations. The animals can also be rendered sensitive to the original antiserum by exhaustive washing. It seems, therefore, that the recovered animals are protected from the action of further antibodies.

*Tetrahymena* contain and excrete several proteases amongst which is an enzyme resembling papain $^{9,10}$ . As papain is known to cleave IgG of various mammalian species to produce Fab fragments $^{11}$ , it seemed possible that the *Tetrahymena* recover from antiserum treatment by specific cleavage of the antibodies by the papain-like enzyme and production of Fab-like fragments capable of binding to the antigens. These bound univalent fragments would then protect the animals without apparently deranging them. If such a mechanism were responsible for the recovery of *Tetrahymena* from antibody agglutination it would be expected that the recovered animals should be sensitive to agglutination by antibodies specific for the immunoglobulin originally used to agglutinate them and from which they had recovered. Furthermore, antibodies specific for the non-antigen binding fragment, or Fc, would not be expected to have an effect on the recovered animals. To test these predictions animals were treated with

specific rabbit anti-*Tetrahymena* serum and allowed to recover. The recovered animals were then washed once and treated with either sheep anti-rabbit total IgG (SaRIgG) or with sheep anti-rabbit Fc (SaRfFc). It was seen that treatment of the recovered animals with SaRIgG resulted in re-agglutination whereas treatment with SaRfFc had no effect. SaRIgG treatment of animals grown in pre-immune rabbit serum or in the absence of serum had no effect. Incubation of the reagglutinated animals again resulted in recovery, showing that sheep IgG is also sensitive to the *Tetrahymena* enzyme(s).

To demonstrate the cleavage of IgG by *Tetrahymena*  $^{14}\text{C}$ -labelled mouse IgG was incubated at  $37^\circ\text{C}$  in the presence of  $10^{-3}\text{M}$  EDTA and  $10^{-3}\text{M}$  dithiothreitol with washed *Tetrahymena* organisms. This treatment did not affect the viability of the organisms. After incubation the samples were analysed by SDS polyacrylamide gel electrophoresis. The gel was then dried and autoradiographed. The results are shown in Fig. 1. It can be seen that the light chain of the IgG was not degraded by the cells. The heavy chain was almost completely lost and replaced by two closely spaced bands which migrated just behind the light chain. The preparation of  $^{14}\text{C}$ -labelled mouse immunoglobulin also contained a small quantity of IgM. It can be seen that the  $\mu$  chain also disappeared after treatment with the cells although it is not known whether Fab was produced from the IgM. Similar results are seen when the immunoglobulins are incubated with *Tetrahymena* culture supernatants.

It seems clear from these observations that *Tetrahymena* of syngen 9 can recover from antibody agglutination by cleavage of the antibodies to produce univalent fragments which remain bound to the antigens and protect the animals from the effects of further antibody treatment. This 'fabulation' process suggests an efficient mechanism by which parasitic protozoa might escape the host immune system. Cell-bound univalent antibodies would be expected to have two effects: they would block antigenic sites from antigen sensitive cells, thus reducing the host's immune response, and they would protect the parasites from circulating antibody. Furthermore, cell-bound Fab would not activate complement.

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1. Beale, G. H. *The Genetics of Paramecium aurelia* (Cambridge University Press, New York, 1954).
2. Takayanagi, T. & Enriques, G. L. *J. Parasitol.* **59**, 644–647 (1973).
3. Allen, S. & Gibson, I. in *Biology of Tetrahymena* (ed. Elliott, A. M.) 307 (Dowden, Hutchinson and Ross, Stroudsburg, Pennsylvania, 1973).
4. Margolin, P., Loefer, J. B. & Owen, R. D. *J. Protozool.* **6**, 207–251 (1959).
5. Juergensmeyer, E. B. *J. Protozool.* **16**, 344–352 (1969).
6. Robertson, M. J. *Pathol. Bact.* **48**, 305–322 (1939).
7. Harrison, J. A. in *Biological specificity and growth* (eds Butler, E. G. et al.) 141–156 (Princeton University Press, Princeton, 1956).
8. Loefer, J. B., Owen, R. D. & Christensen, E. J. *Protozool.* **5**, 209–217 (1958).
9. Viswanatha, T. & Liener, I. *Arch. biochem. Biophys.* **61**, 410–421 (1956).
10. Dickie, N. & Liener, I. *Biochem. biophys. Acta* **64**, 52–59 (1962).
11. Porter, R. R. *Biochem. J.* **73**, 119–126 (1959).
12. Laemmli, U. K. *Nature* **227**, 680–685 (1970).

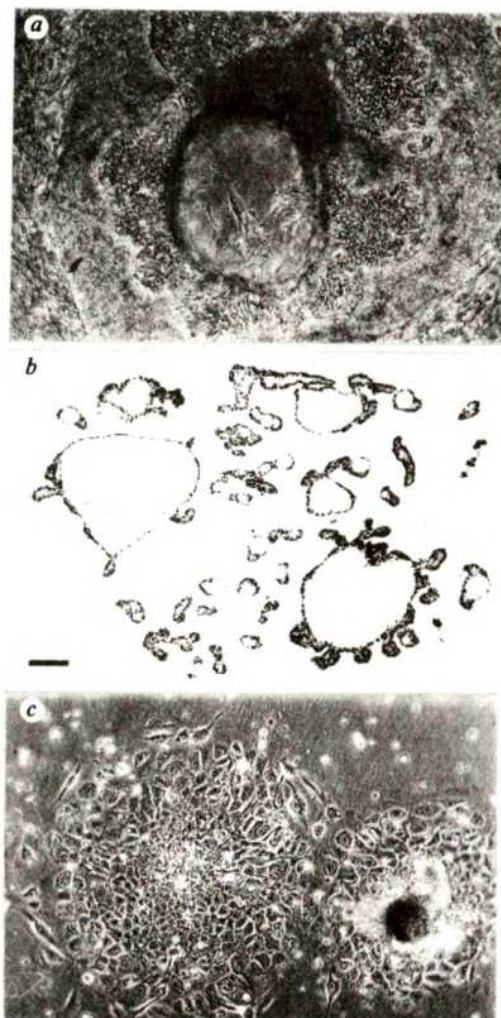
## Isolation of a human teratoma cell line which expresses F9 antigen

THE F9 antigen, defined by antisera raised in syngeneic mice against pluripotent embryonal carcinoma cells, is present on early mouse embryos, spermatozoa and male germinal cells, but not on adult somatic tissues $^{1,2}$ . This antigen, thought to be coded by gene(s) located at, or linked to, the developmentally important *T/t* complex, may play a part in early embryogenesis $^{3-5}$ . This idea is supported by the fact that the antigen seems to have been conserved during mammalian evolution. Anti-F9 activity is absorbed by sperm of several

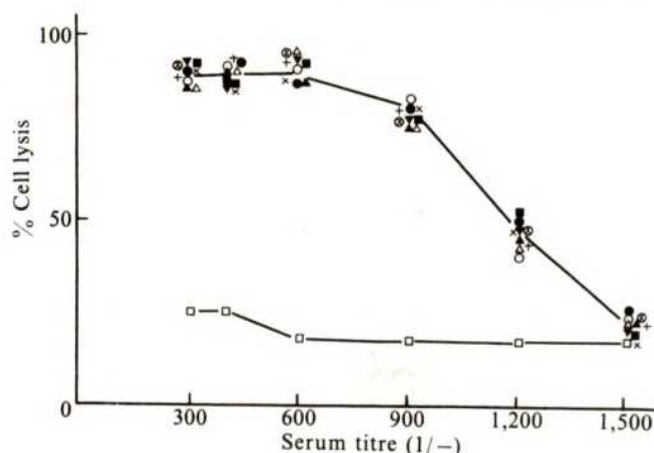


species including man, rat, rabbit and bull<sup>3-7</sup> and there is evidence for its presence on the morulae of rabbit, rat and cow, and on human foetal testicular cells<sup>2,5</sup>. The cross reaction of anti-F9 with human sperm suggested that undifferentiated stem cells in human teratocarcinomas might also carry F9 on their surface, and we report here the isolation of human teratocarcinoma cell line which expresses F9 antigen.

The cell line, known as SuSa, was isolated from a 30-yr-old male with a malignant testicular teratoma, intermediate grade B with extensive necrosis. Sections of the primary tumour had areas of undifferentiated cells in convoluted epithelial sheets, networks and vesicles, surrounded by simple tumour mesenchyme; limited areas of more differentiated cells, including keratinised and glandular epithelium and cartilage were also present. The patient had



**Fig. 1** Morphology of SuSa cells in culture. Pieces from different sites in the primary tumour were minced finely with scissors and placed in a 90-mm dish containing a monolayer of  $3 \times 10^6$  STO mouse feeder fibroblasts<sup>8</sup> in Dulbecco's modified Eagle's medium (MEM) with 10% calf and 10% foetal bovine serum. After about 5 d small patches of closely packed, homogeneous cells were seen, and after 2 weeks these were subcultured with 0.05% trypsin-0.5 mM EDTA in phosphate-buffered saline (PBS), pH 7.2. The cells have a very low plating efficiency (about 4%) even on feeder layers. Feeder layers were prepared by treating STO cells before plating out with mitomycin C ( $20 \mu\text{g ml}^{-1}$ ) for 2-3 h and 6,000-10,000 rad X rays. *a*, A vesicle in the centre of a colony of cells growing at high density on a feeder layer. Phase contrast microscopy; bar represents 100  $\mu\text{m}$ . *b*, Section through a chain of vesicles detached from a high density culture. Stained with haematoxylin and eosin, bar represents 50  $\mu\text{m}$ . *c*, Cells growing out from a vesicle which had attached to a tissue culture dish in the absence of feeder fibroblasts; bar represents 100  $\mu\text{m}$ .



**Fig. 2** Absorption of anti-F9 activity by SuSa and other human cell lines and mouse STO fibroblasts. Anti-F9 serum was elicited by hyperimmunisation of male 129/Sv mice with irradiated cells of the mouse teratocarcinoma line F9-41<sup>1</sup>, and was absorbed before use with cells of the parietal yolk sac line PYS2 and with human fibroblast (skin) cells (v/v, serum diluted 1/5 in Hanks medium containing 4% heat-inactivated  $\lambda$  globulin-free foetal bovine serum (IPT), for 1 h at 0 °C). Such absorbed sera are active against F9 cells in both the microcytotoxicity test (90-95% of cells killed at titres of 1/2400-1/3200) and the indirect immunofluorescence assay (90-100% of the F9 cells labelled at dilutions up to 1/400). Absorptions were made with 100  $\mu\text{l}$  of anti-F9 sera diluted 1/300 in Hanks medium + 4% IPT. After absorption with  $8 \times 10^6$  SuSa cells or  $1 \times 10^7$  cells of all the other cell lines tested at 4 °C for 1 h, the cells were removed by centrifugation and the residual cytotoxic activity was tested on F9 cells using serial dilutions in the presence of absorbed rabbit serum as a source of complement<sup>1</sup>. ●, Unabsorbed serum; □, absorbed on SuSa; △, absorbed on Burkitt's lymphoma<sup>9</sup>; ▲, absorbed on an intestinal cell line (American Type Culture Collection CCL6); ×, absorbed on an epidermal larynx tumour (ATCC CCL23); +, absorbed on HeLa (ATCC CCL2); ▼, absorbed on melanomas<sup>10</sup>; ⊗, absorbed on a rhabdomyosarcoma (ATCC CCL136); ○, absorbed on STO mouse fibroblasts.

multiple metastases and high levels of human chorionic-gonadotropin in the urine. HLA typing of the patient's peripheral blood lymphocytes was A1, A3, B5, Bw17 and possibly Cw3.

The cells are maintained on feeder layers of mouse fibroblasts and have a low ratio of cytoplasm to nucleus, and prominent nucleoli. They grow as flat colonies of densely packed cells which gradually pile up at the edges and centre, forming chains of simple epithelial vesicles which float off into the medium (Fig. 1a). Similar vesicles are produced when SuSa cells are subcultured in the absence of feeder fibroblasts. When floating vesicles attach to the tissue culture dish, the cells migrate out and become flattened (Fig. 1c), but they do not continue to grow when subcultured in the absence of feeder fibroblasts. In sections of fixed and stained material (Fig. 1b) SuSa cells closely resemble the undifferentiated epithelial cells of the primary tumour. In the electron microscope they show very little endoplasmic reticulum but have some microvilli and lipid droplets, and are joined by junction complexes. No virus particles have been seen.

Approximately 10 passages after its isolation the cell line had a modal chromosome number of 58, and its human origin was confirmed by the presence of metacentric chromosomes. Quinacrine mustard staining showed one Y chromosome in most spreads.

Absorption of anti-F9 serum with  $8 \times 10^6$  SuSa cells removed cytotoxic activity towards F9 cells (Fig. 2). Further experiments showed that as few as  $3 \times 10^6$  SuSa cells could remove most of the anti-F9 activity of the sera in the conditions described in Fig. 2. Anti-F9 activity was not absorbed by the STO cells used as feeders, nor by any of the human cell lines tested—HeLa, two Burkitt tumours,

two melanomas, an epidermal carcinoma of the larynx and an embryonal rhabdomyosarcoma.

In immunofluorescence studies (Table 1) anti-F9 serum reacted strongly with about half of the SuSa plus feeder cell population, but marked neither human nor mouse fibroblasts. No fluorescence labelling was observed after absorption of the serum by F9 cells or human sperm. Because mouse embryonal carcinoma cells which are F9 positive do not express H2 antigens or  $\beta_2$  microglobulin<sup>8</sup> we tested SuSa cells for the products of the major histocompatibility HLA locus in man. We found that only a very small percentage of SuSa cells was labelled by specific HLA-A1 or B5 antisera. The percentage of cells that bound anti-human  $\beta_2$  microglobulin in serum correlated well with the percentage of HLA positive cells. Together with the absorption studies, these results show that cultured SuSa cells express an antigen which cross-reacts with the F9 antigen on mouse embryonal carcinoma cells and human sperm, and that this expression is not due to the presence of STO feeder cells. Furthermore, most cells expressing the F9 antigen do not express HLA antigens, and judging by the presence of HLA antigens, only a minority of the human cells in the cultures seem to be differentiated.

Table 2 shows the results of double immunofluorescence labelling. When anti-F9 and a specific anti-HLA-B5 serum were used simultaneously all F9 positive cells were HLA-B5 negative, and *vice versa*. The result was similar when a rabbit anti-HLA serum was used in combination with anti-F9, suggesting mutual exclusion of F9 and HLA antigens on the SuSa cells. Double labelling with anti-F9 and an anti-human  $\beta_2$  microglobulin serum revealed that all F9 positive cells were  $\beta_2$  microglobulin negative. The human nature of the F9 positive cells in the SuSa population was confirmed by

**Table 2** Double marking immunofluorescence studies on cell line SuSa

First antisera	Second antisera	% Positive cells
Anti-F9 + goat anti-mouse FITC	TRITC-anti-HLA-B5	48 F9 <sup>+</sup> HLA <sup>-</sup> , 0 F9 <sup>+</sup> HLA <sup>+</sup> 47 F9 <sup>-</sup> HLA <sup>-</sup> , 5 F9 <sup>-</sup> HLA <sup>+</sup>
Anti-F9 + goat anti-mouse FITC	Rabbit anti-HLA + goat anti-rabbit TRITC	44 F9 <sup>+</sup> HLA <sup>-</sup> , 0 F9 <sup>+</sup> HLA <sup>+</sup> 48 F9 <sup>-</sup> HLA <sup>-</sup> , 8 F9 <sup>-</sup> HLA <sup>+</sup>
Anti-F9 + goat anti-mouse FITC	Rabbit anti-human $\beta_2$ microglobulin + goat anti-rabbit TRITC	50 F9 <sup>+</sup> $\beta_2$ m <sup>-</sup> , 0 F9 <sup>+</sup> $\beta_2$ m <sup>+</sup> 40 F9 <sup>-</sup> $\beta_2$ m <sup>-</sup> , 10 F9 <sup>-</sup> $\beta_2$ m <sup>+</sup>
Anti-F9 + goat anti-mouse FITC	Rabbit anti-human fibroblast + goat anti-rabbit TRITC	0 F9 <sup>+</sup> Man <sup>-</sup> , 32 F9 <sup>+</sup> Man <sup>+</sup> 24 F9 <sup>-</sup> Man <sup>-</sup> , 44 F9 <sup>-</sup> Man <sup>+</sup>

See Table 1. Anti-human fibroblast serum was raised in rabbits immunised with three injections of human skin fibroblasts from a single donor. The serum was absorbed on mouse liver and spleen and on the mouse fibroblast line STO to render it specific. This serum is active on both HLA positive and HLA negative (Daudi) human cell lines. It was used at a dilution of 1/2,000 and revealed with goat anti-rabbit TRITC or FITC.

a double labelling experiment using anti-F9 and a pre-absorbed rabbit anti-human fibroblast serum. All F9 positive cells were labelled by this serum.

The studies described so far involved dissociated single cells. When intact aggregates were tested for F9 using methods applied to preimplantation embryos<sup>4</sup>, about 30% of them were marked by anti-F9 antiserum. When aggregates were dissociated before exposure to antiserum, 50–55% of the cells were F9 positive while about 80% were marked by anti-human fibroblast serum.

Several lines of evidence suggest that SuSa cells are equivalent to the undifferentiated stem cells of human testicular teratocarcinomas. First, most of them resemble morphologically the undifferentiated epithelial cells of the original tumour and other testicular teratomas<sup>11–13</sup>. Second, most of them express an antigen which cross-reacts with the F9 antigen on mouse embryonal carcinoma cells, and third, they do not express, or have available for reaction, products of the major histocompatibility HLA locus or  $\beta_2$  microglobulin. Although a few HLA positive cells are found in SuSa cultures we have no conclusive morphological evidence that the cells differentiate *in vitro*, nor can we exclude the possibility that the cells are differentiating into cytotrophoblast, which may not express HLA antigens. Proof of the tumorigenicity and stem cell nature of SuSa cells would be unambiguous if single cell clones gave rise to differentiated tumours in nude mice. But when eight nude mice were each injected subcutaneously with  $1 \times 10^6$  SuSa cells, no tumours developed after 2 months. In earlier experiments, cells taken directly from five primary testicular teratomas and one ovarian teratoma were tested for F9 by absorption and found to be negative (Buc. G. Gachelin and M.F. unpublished results). This may have reflected the small proportion of undifferentiated stem cells in the tumours tested but we cannot formally exclude the possibility that SuSa cells have acquired F9 expression *in vitro*.

Our results and those of Holden *et al.* (see following communication)<sup>14</sup> who find F9 on another line of human teratocarcinoma cells, Tera I, raise the possibility of looking for circulating autoantibodies in patients with testicular teratoma (and possibly seminoma), using cell lines such as SuSa, Tera I and F9 as targets.

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**Table 1** Immunofluorescence studies on cell line SuSa, human fibroblast HLA-B5 and mouse fibroblast STO

Antisera used	% Cells fluorescing		
	Human teratoma SuSa	Human fibroblast HLA-A1 or HLA-B5	Mouse fibroblast STO
Anti-F9 + goat anti-mouse FITC	48,46,44,50	0	0
Anti-F9 preabsorbed on F9 + goat anti-mouse FITC	2	0	0
Anti-F9 preabsorbed on human sperm + goat anti-mouse FITC	4	0	0
Anti-HLA-B5 TRITC	6,5	80	0
Anti-HLA-A1 FITC	3,4	100	0
Rabbit anti-HLA + goat anti-rabbit TRITC	8	100	0
Rabbit anti-human $\beta_2$ microglobulin + goat anti-rabbit TRITC	10,7	90	0

$5 \times 10^6$  cells were centrifuged in a microfuge (Beckman Instruments Palo Alto). The pellet was resuspended in 50  $\mu$ l of antisera at an appropriate dilution in MEM+4% IPT and kept at 4 °C. After 45 min the cells were washed with the same medium and suspended in an anti-IgG globulin labelled either with fluorescein isothiocyanate (FITC) (Hyland) or with tetramethyl rhodamine isothiocyanate (TRITC). After 45 min at 4 °C, the cells were washed three times, resuspended in PBS pH 7.2, spread, air dried, fixed with methanol, then mounted in glycerol: PBS (80/20; v/v). Preparations were observed using a Leitz Orthoplan microscope. Appropriate specificity controls (normal and absorbed immune sera) were performed. Anti-F9 serum was used at a dilution of 1/80 and revealed by a goat-anti-mouse IgG and IgM, labelled with FITC. In the F9 controls, absorptions were made v/v with F9 antisera and cells for 1 h at 4 °C. Anti-HLA-A1 and anti-HLA-B5 were obtained from multiparous women. Anti-HLA-A1 was labelled directly with FITC. Anti-HLA-B5 was labelled directly with TRITC. Both sera were used at 1/25 dilutions and had been absorbed on mouse STO cells before use. The rabbit anti-HLA 5996-2 and the rabbit anti human  $\beta_2$  microglobulin sera (5895-21) raised against purified preparations of papain-solubilised HLA antigen and of human  $\beta_2$  microglobulin were given by N. Tanigaki. They were both preabsorbed with the Daudi lymphoma and STO cells before use. They were revealed with goat anti-rabbit IgG labelled with TRITC.



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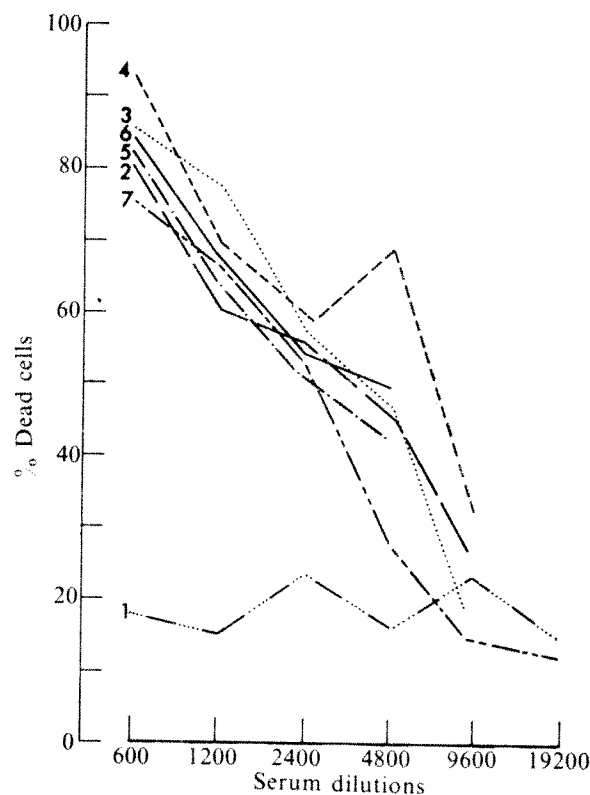
1. Artzt, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 2988–2992 (1973).
2. Gachelin, G., Fellous, M., Guenét, J.-L. & Jacob, F. *Devl Biol.* **50**, 310–320 (1976).
3. Artzt, K., Bennett, D. & Jacob, F. *Proc. natn. Acad. Sci. U.S.A.* **71**, 811–814 (1974).
4. Kemler, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4080–4084 (1977).
5. Jacob, F. *Immun. Rev.* **33**, 3–32 (1977).
6. Buc-Caron, M.-H., Gachelin, G., Hofnung, M. & Jacob, F. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1730–1733 (1974).
7. Fellous, M., Gachelin, G., Buc-Caron, M.-H., Dubois, P. & Jacob, F. *Devl Biol.* **41**, 331–337 (1974).
8. Hogan, B. L. M. *Nature* **263**, 136–137 (1976).
9. Clements, G., Klein, G., Zeulhen, J. & Povey, S. *Somatic Cell Genet.* **2**, 309–324 (1976).
10. Janiaud, P., Le Calvez, J. & Aubert, Ch. *Analyt. Derm. Syph. Paris* **100**, 536–539 (1973).
11. Willis, R. A. *Pathology of Tumours*, 3rd edn. (Butterworths, London, 1960).
12. Dixon, F. J. & Moore, R. A. *Cancer* **6**, 427–454 (1953).
13. Mostafaei, F. K. *Rec. Results Cancer Res.* **60**, 176–195 (1977).
14. Holden, S. *et al. Nature*, **270**, 518–520 (1977).

## Human and mouse embryonal carcinoma cells in culture share an embryonic antigen (F9)

THE embryonic membrane antigen F9 has been detected on several lines of mouse embryonal carcinoma (EC) cells as well as on mouse embryos and sperm, but not on any other adult cells tested<sup>1</sup>. F9 or an antigenically similar substance has been demonstrated on the morula and/or sperm of several mammalian species<sup>2</sup> including man<sup>3</sup>, but has been undetectable in all non-mammalian species tested<sup>2</sup>. Although no function for this antigen has been identified, its restriction to embryonic and germ cells suggests an important developmental role. This possibility is enhanced by the apparent conservation of F9 in the course of evolution. We now report the serological detection of F9 on human EC, together with our inability to detect  $\beta_2$  microglobulin.

We investigated two established tissue culture lines of human EC cells for the presence of F9 antigen. These lines (Tera I, Tera II) were cultured from the pulmonary metastases of two men with testicular teratocarcinoma<sup>4</sup>. *In vitro*, these EC cells seem morphologically similar to murine EC cells and do not exhibit visible differentiation in standard growth conditions. Attempts are in progress to characterise these lines by growing them in athymic mice. Using mouse anti-F9 antisera, we have shown in complement-mediated cytotoxicity assays and by absorption that F9 antigen is detectable on Tera II but not on Tera I (Figs 1 and 2). F9 could not be detected by similar techniques on normal human fibroblasts or on three human lines derived from lung, intestinal and skin tumours. It is interesting that in direct cytotoxicity tests, Tera II was almost as sensitive to anti-F9 antisera as its murine counterpart.

Immunoperoxidase studies confirmed the presence of F9 antigen on Tera II cells and also showed that F9 can be detected on a small fraction of the Tera I cell population (Fig. 3).



**Fig. 1** Complement-mediated cytotoxic activity of anti-F9 serum on F9 cells before absorption (1), after double absorption with human EC cells, Tera II (1), Tera I (3), human melanoma, MalME (4), human intestinal tumour HT 29 (5), human lung tumour SKMEM-1 (6) and murine yolk sac carcinoma PYS (7). F9 cells originated from Steven's OTT6050 teratocarcinoma; human cells were isolated and supplied by Føgh<sup>4</sup>. All cells were cultivated at 37 °C in 12% CO<sub>2</sub> in air as a monolayer on plastic Petri dishes in Dulbecco's modified Eagle's medium containing 15% foetal calf serum and 1% penicillin/streptomycin. F9 but not human cells were grown on a 0.1% gelatin layer. The preparation of antisera, complement and cells as well as the details of the cytotoxicity test are identical to the technique previously described<sup>1</sup> except that double absorptions performed at 4 °C for 45 min each utilising a 1:2 ratio of cell volume to serum volume were required for Tera II to remove anti-F9 activity. The absorptions were performed at a serum concentration of 2 doubling dilutions below the titre. Lines 1–3 represent the averages of three to nine independent experiments. Lines 4–7 represent single experiments. All complement and cell controls were between 10 and 22% dead.

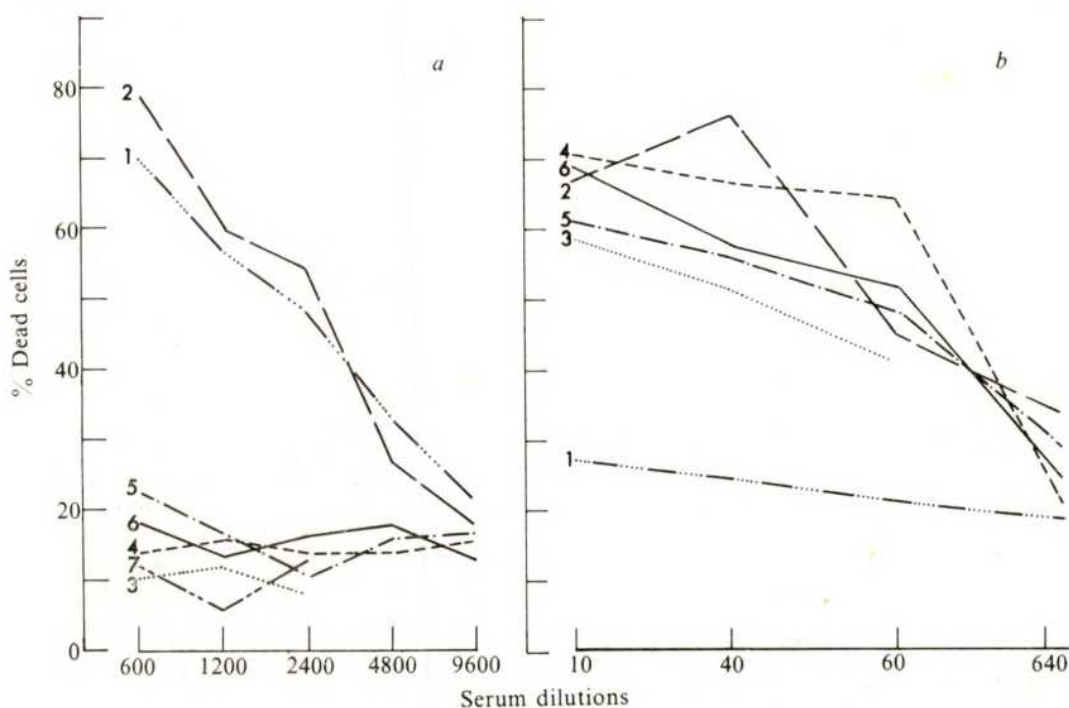
Both EC and early embryo cells contain large quantities of alkaline phosphatase which decline with the onset of differentiation<sup>5</sup>. Although the function of this enzyme is unknown, it is a useful marker for cellular differentiation. We tested Tera I and Tera II for the presence of alkaline phosphatase by the method of Burstone<sup>6</sup>. Our findings that Tera II cells contained high levels of alkaline phosphatase plus F9 antigen while most Tera I cells lacked F9 antigen and had low alkaline phosphatase could be explained if most Tera I cells are more differentiated than Tera II.

Because mouse EC or embryonic cells that express F9 antigen lack H-2 (ref. 7), and H-2 becomes detectable with the onset of differentiation as F9 disappears<sup>8</sup>, we tested indirectly for the presence of HLA antigen on Tera II cells. Because we did not know the HLA type of the patients from whom these cells derive, we tested for the presence of  $\beta_2$  microglobulin, with which the HLA antigens are invariably associated. Complement-mediated cytotoxicity tests and absorption showed that Tera II did not express  $\beta_2$  microglobulin, while Tera I and other human cell lines tested did (Figs 2 and 4). This suggests not only that Tera II lacks HLA but also that it lacks the human counterpart of other mouse antigens such as TL and QA-2, which are also associated with  $\beta_2$  microglobulin in the membrane.

Two observations made in patients with testicular cancer suggest that human teratocarcinoma does not stimulate the host



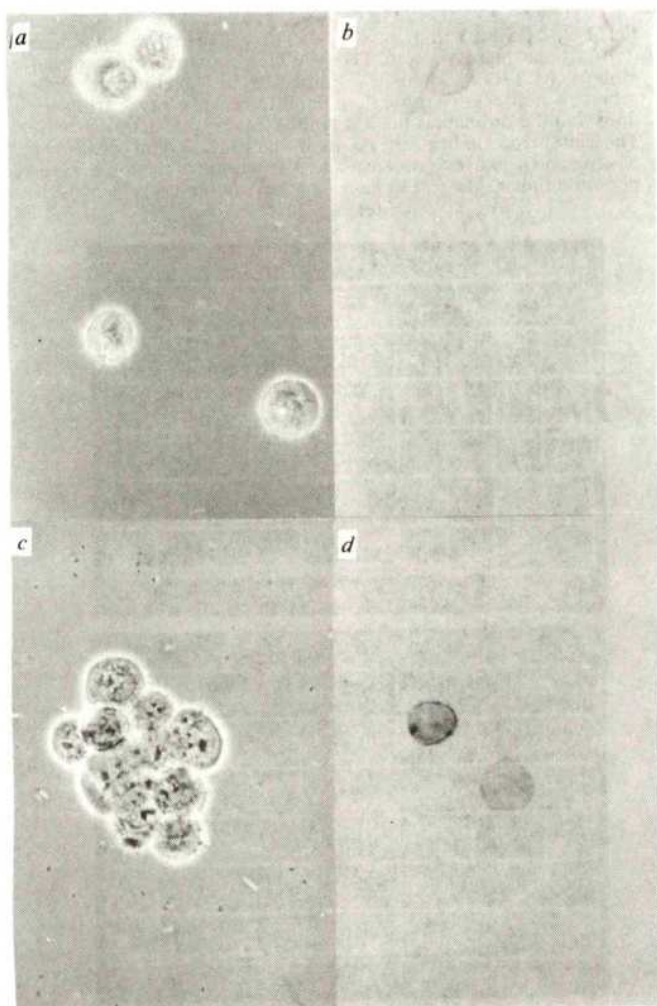
**Fig. 2** *a*, Direct complement-mediated cytotoxic activity of murine anti-F9 serum on Tera II (1), F9 (2), SKMEM-1 (3), Ma1ME (4), Tera I (5), human fibroblasts (6) and HT29 (7). Lines 1-4 represent the averages of two to nine independent experiments. Lines 5-7 represent single experiments. Complement controls averaged 23.8% dead and were all below 30%. Cell controls averaged 16.4% dead and were all below 25%. *b*, Direct complement-mediated cytotoxic activity of rabbit anti-human  $\beta_2$  microglobulin (DAKO-immunoglobulins code no. 10-U72, Dakopatts, Denmark) on Tera II (1), fibroblasts (2), Tera I (3), HT-29 (4), SKMEM-1 (5) and Ma1ME (6). Lines 1, 2 and 6 represent averages of two independent experiments. Lines 3-5 represent single experiments. Complement, serum and cell controls ranged from 9 to 28% dead and averaged 18.4%.



cellular immune response. First, unlike patients with virtually all other forms of cancer, those with testicular tumours in advanced stages demonstrate a grossly intact cellular immune system when measured by their ability to respond by delayed cutaneous

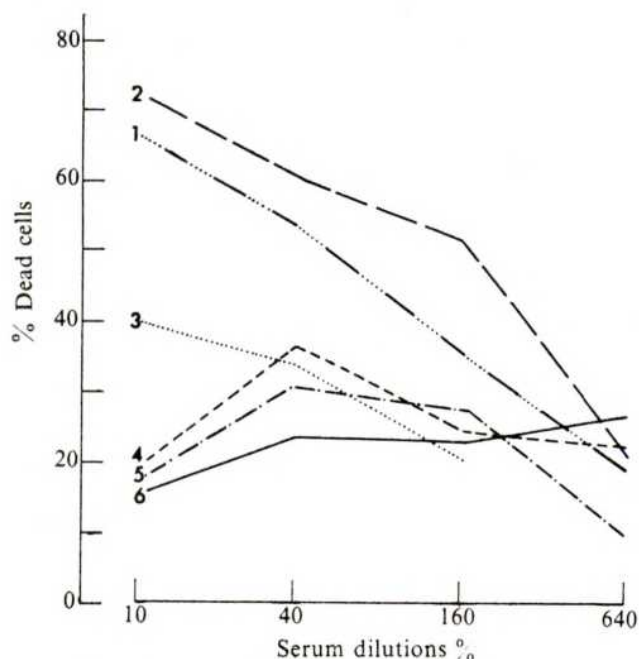
hypersensitivity to intradermal antigens such as dinitrochlorobenzene<sup>9</sup>. Second, unlike the case of patients with various cancers tested, the immunomorphological features of the regional lymph nodes in testicular cancer have not been correlated successfully with prognosis<sup>10</sup>. The lack of HLA on teratocarcinoma may explain these anomalies. For example, the absence of H-2 on F9 cells apparently prevents them from serving as target cells either for allogeneic cytotoxic T cells immunised against spleen cells syngeneic to F9, or for syngeneic T cells sensitised to trinitrophenyl- or virus-modified spleen cells<sup>11,12</sup>. It has been suggested that the phenotypic expression of H-2 genes by the target cell is necessary for cell-mediated immunity<sup>12,13</sup>. Thus T cells in regional lymph nodes of human patients may be unable to see EC cells because of their lack of HLA expression. This hypothesis, if correct, could have direct impact on plans for immunotherapy of this disease.

Because embryonic antigens (F9) stimulate a humoral or 'autoantibody' response in syngeneic systems, and human teratocarcinomas express F9 antigen, our current efforts are directed toward the detection of anti-F9 autoantibody in the sera of



**Fig. 3** Immunoperoxidase study of anti-F9 serum on human teratoma cell lines.  $1-2 \times 10^6$  cells were incubated for 60 min at  $+4^\circ\text{C}$  in 0.1 ml anti-F9 serum diluted 1:50 in Hanks medium supplemented with 5% heat-inactivated  $\gamma$ -globulin-free foetal calf serum (Gibco). Cells were washed three times in the same medium and resuspended in 0.1 ml of a  $65 \mu\text{g ml}^{-1}$  solution of peroxidase-labelled sheep Fab anti-mouse immunoglobulins (Institut Pasteur) for another 1 h at  $+4^\circ\text{C}$ . After three washings in medium, cells were fixed in 2% glutaraldehyde in phosphate-buffered saline (PBS), further washed in PBS and stained for peroxidase activity in diaminobenzidine (50 mg per 100 ml in Tris-HCl, pH 7.5) and 0.01%  $\text{H}_2\text{O}_2$  for 3 min at room temperature. *a*, Tera II cells incubated in anti-F9 antiserum, phase contrast micrograph. *b*, Tera II cells incubated in anti-F9 antiserum. A discrete labelling of the cell membrane was detectable in 80% of the cells. No labelling was present when anti-F9 serum had been previously absorbed on F9 cells. Absorption of anti-F9 serum on PYS cells did not alter the labelling observed with unabsorbed serum ( $\times 350$ ). *c*, Tera I cells incubated in anti-F9 serum. Most of the cells were unlabelled, but about 15% showed a membrane labelling sometimes stronger than that on Tera II cells. This labelling was no longer detectable with anti-F9 serum absorbed on F9 cells ( $\times 350$ ). When Ma1ME cells were incubated with the same anti-F9 serum, some cell membrane labelling could be detected. This, however, was not removed by absorption of anti-F9 serum on F9 cells.





**Fig. 4** Complement-mediated cytotoxic activity of rabbit anti-human  $\beta_2$  microglobulin on human fibroblasts before absorption and (2) after single absorption with Tera II (1), Tera I (3), HT 29 (4), SKMEM-1 (5) and Ma1 ME (6). Lines 1 and 3 represent the averages of two independent experiments. Lines 3-6 represent single experiments. All complement, serum and cell controls varied between 8 and 28% dead.

patients with testicular cancer. If present, this activity could serve as a highly specific biological marker of tumour activity.

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1. Artzt, K. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **70**, 2988 (1973).
2. Jacob, F. *Immun. Rev.* **33**, 3 (1977).
3. Fellous, M., Gachelin, G., Buc-Caron, M. H., Dubois, P. & Jacob, F. *Dev. Biol.* **41**, 331 (1975).
4. Fogh, J. & Trempe, G. in *New Human Tumor Cell Lines* (ed. Fogh, J.) 115-119 (Plenum, New York and London, 1975).
5. Bernstine, E. G., Hooper, M. L., Grandchamp, S. & Ephrussi, B. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3889 (1973).
6. Lillie, R. D. *Histopathologic Technique and Practical Histochemistry* 314 (McGraw-Hill, New York, 1965).
7. Artzt, K. & Jacob, F. *Transplantation* **17**, 633 (1974).
8. Nicolas, J. F., Dubois, P., Jakob, H., Gaillard, J. & Jacob, F. *A. Microbiol., Institut Pasteur* **126A**, 3 (1975).
9. Schellhammer, P. F., Bracken, R. B., Bean, M. A., Pinsky, C. M. & Whitmore, W. F. *Cancer* **38**, 149 (1976).
10. Jewett, M. A. S., Hadju, S. I., Good, R. A. & Whitmore, W. F. *Br. J. Urol.* **49**, 335 (1977).
11. Forman, J. & Vitetta, E. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3661 (1975).
12. Zinkernagel, R. M. & Oldstone, M. B. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3666 (1976).
13. Doherty, P. C., Solter, D. & Knowles, B. B. *Nature* **266**, 361 (1977).

## Murine pluripotential stem cells lack Ia antigen

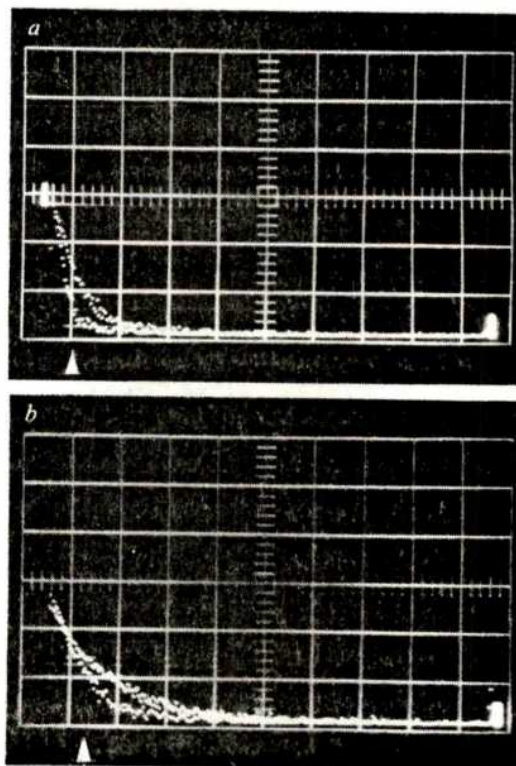
THE Ia (I region associated) antigens of the mouse were first described as differentiation antigens of B lymphocytes<sup>1,2</sup>. Subsequently, similar antigens, determined by the same or closely linked genes, have been found on macrophages<sup>3</sup>, on some T cells<sup>4</sup> and on stable products released by T cells<sup>5</sup>. Immunoprecipitated Ia antigen is a dimeric molecule, consisting of two dissimilar polypeptides<sup>6</sup>. The alloantigenic polymorphism of the structure is attributed to variation in the larger (33,000 molecular weight)

subunit. Evidence has accumulated suggesting that Ia antigens are involved in immunological recognition and/or cell interactions<sup>8,9</sup>.

Structures with similar biochemical properties have been identified on human cells and specific hetero- and allo-antisera against these Ia-like antigens are available<sup>10-14</sup>. The distribution of the human 'Ia-like' material on normal cells closely parallels that found in mice. Cells in chronic lymphocytic leukaemia and B type lymphomas have been found to be positive<sup>11-16</sup>. Unexpected and intriguing results were obtained, however, when the distribution of these antigens on leukaemic and foetal cells was studied. Most leukaemic lymphoblasts in patients with the common, non-T, non-B type of acute lymphoid leukaemia (ALL), myeloblasts in patients with acute myelocytic leukaemia (AML) and blast cells found in patients with chronic myeloid leukaemia in blast crisis (CML-BC) expressed a Ia-like antigen which was completely cross-reactive with B cell Ia (refs 14-16). More mature myeloid cells (myelocytes and granulocytes) on the other hand were Ia negative<sup>16,17</sup>. Ia positive, membrane immunoglobulin negative, lymphoblasts, myeloblasts and in some cases promyelocytes, were also found in non-leukaemic adult and foetal bone marrow cells<sup>16,17</sup>. On this basis it was suggested that 'Ia-like' antigens on blast cells in acute leukaemia may reflect their origin from Ia positive haematopoietic stem cells<sup>16,17</sup>.

A direct assay for cells which serve as a precursor to both lymphocytes and granulocytes is not available in man. These pluripotential stem cells can, however, be quantified in the mouse. Transfer of limited numbers of haematopoietic cells into irradiated mice results in the formation of discrete nodules in the spleen<sup>18</sup>. These spleen colonies are the product of the clonal proliferation of pluripotential stem cells (CFU<sub>s</sub>). We have examined the effects of antisera to Ia antigens on the cells which give rise to spleen colonies.

**Fig. 1** FACS histogram analysis of Ia positive cells from mouse (CBA) bone marrow. *a*, A.TH  $\alpha$  A.TL compared with normal mouse (A.TH) serum; *b*, rabbit  $\alpha$  'Ia' compared with rabbit  $\alpha$  mouse Ig. The abscissa is fluorescence intensity in arbitrary units and the ordinate is the log of the frequency of detected cells. The white arrows indicate the points at which the division between Ia positive and negative cells were made. The settings of the sorter were: photomultiplier tube 600 V, laser, 400 mW, fluorescence gain 16:1, scatter gain 2/0.5.



The results in treating mouse bone marrow cells with polyvalent alloantiserum against Ia (A.TH  $\times$  A.TL) and complement are shown in the first experiment in Table 1. The anti-Ia serum did not reduce the number of colonies found. Line 3 of the Table shows that the CFU of CBA mice can be killed by antibody (rabbit anti-mouse brain) and complement in the conditions used. Since the failure of alloantiserum to kill is not proof of the absence of antigen, we used the fluorescence-activated cell sorter (FACS I) to separate Ia<sup>+</sup> and Ia<sup>-</sup> cells from mouse bone marrow and tested these cells for their ability to form spleen colonies. To prevent the complement mediated destruction of antibody-coated cells after their injection into the irradiated recipients the cells were incubated at 37 °C for 1 h in tissue culture medium RPMI1640 supplemented with 2% heat inactivated foetal bovine serum. This treatment effectively removes the antibody both as judged by fluorescence microscopy and by the recovery of CFU<sub>(s)</sub> from cells treated with rabbit anti-mouse brain (Fab<sub>2</sub>) and fluoresceinated goat anti-rabbit Ig (lines 10 and 11, experiment 2, Table 1). 14.1% of the total are Ia positive on the basis of the sorter analysis (compared to 6.0% when stained with normal mouse serum) as shown in Fig. 1a. Both the positive cells and the negative cells were collected and injected into irradiated recipients. The results are shown in experiment 2, Table 1. Almost all of the CFU<sub>(s)</sub> of C<sub>3</sub>H bone marrow was recovered in the Ia<sup>-</sup> fraction. The apparent activity of the Ia<sup>+</sup> fraction (line 8) can be accounted for by

**Table 1** Effect of anti-Ia alloantiserum on CFU<sub>(s)</sub> obtained from mouse bone marrow

Expt 1: negative selection with anti-Ia antiserum and complement

Antiserum	Volume per 10 <sup>6</sup> cells ( $\mu$ l)	Spleen colonies per 10 <sup>5</sup> cells injected	
1. Normal A.TH serum	50	17.4 $\pm$ 2.6	(14)
2. A.TH $\times$ A.TL	50	16.3 $\pm$ 3.5	(12)
3. Normal rabbit serum	20	15.8 $\pm$ 3.1	(5)
4. Rabbit anti-mouse brain	20	2.2 $\pm$ 1.7	(5)
5. No cells transferred	—	0.3 $\pm$ 0.1	(18)

Expt 2: Positive selection using FACS to obtain Ia<sup>+</sup> cells

Cells transferred	Number	Spleen colonies	
6. Unfractionated CBA bone marrow	100,000	18.2 $\pm$ 2.1	(9)
7. Ia negative fraction	100,000	15.6 $\pm$ 3.9	(8)
8. Ia positive fraction	50,000	5.8 $\pm$ 1.6	(4)
	15,000	1.7 $\pm$ 1.5	(5)
9. Unfractionated + NRS	100,000	14.0 $\pm$ 2.8	(5)
10. Unfractionated + R anti-MB + C	100,000	4.2 $\pm$ 2.6	(5)
11. Unfractionated + R anti-MB + goat anti-R1g incubated at 37 °C for 1 h	100,000	14.7 $\pm$ 4.0	(5)
12. No cells transferred	0	0.4 $\pm$ 0.2	(5)

Recipient mice (CBA in expt 1, C<sub>3</sub>H in expt 2) were irradiated with 750 R from a <sup>60</sup>Co source and injected intravenously with bone marrow cells suspended in tissue culture medium. The animals were killed 9 d later and the spleens removed and fixed in Bouins solution. The spleen colonies visible on the surface were counted under a dissecting microscope at 25 $\times$  magnification. In Expt 1, 10<sup>6</sup> bone marrow cells were incubated at 4 °C with 20–50  $\mu$ l of antiserum in a total volume of 250  $\mu$ l. After 1 h they were washed twice and then incubated for 30 min at 37 °C with guinea pig serum diluted 1:3. The cells were then washed twice, resuspended in media and 10<sup>5</sup> cells injected into each mouse. A.TH  $\times$  A.TL, prepared by Dr I. McKenzie, was obtained by Dr Marc Feldmann and had a titre of  $\sim$  1:50 against CBA spleen cells. R  $\times$  MB was prepared as previously described and had a titre of 1:200 (ref. 19). It had been absorbed with mouse red blood cells, liver and cortical thymocytes from CBA mice. In expt 2, 10<sup>7</sup> cells were incubated with 100  $\mu$ l of antiserum in a total volume of 250  $\mu$ l at 4 °C for 30 min, washed twice and then incubated for 30 min with 50  $\mu$ l of a 1  $\mu$ g ml<sup>-1</sup> solution of the fluoresceinated (Fab<sub>2</sub>) fraction prepared by pepsin treatment of the total immunoglobulins of rabbit anti-mouse Ig serum. They were then washed twice and passed through the FACS. All of the FACS-passed and control cells were adjusted to 10<sup>6</sup> cells ml<sup>-1</sup> and incubated at 37 °C for 1 h before being injected into the irradiated mice.

\*Values are means  $\pm$  s.d.; number of tests performed is given in parentheses.

**Table 2** Effect of heterologous antiserum to mouse Ia on the CFU<sub>(s)</sub> obtained from mouse bone marrow

Expt 1: negative selection with anti-Ia and complement

Antiserum	Volume per 10 <sup>6</sup> cells ( $\mu$ l)	Spleen colonies per 10 <sup>5</sup> cells injected	
1. Normal rabbit serum (pre bleed)	50	14.8 $\pm$ 1.6	(5)
2. 'Anti-Ia'	50	13.5 $\pm$ 2.8	(5)
3. 'Anti-Ia' absorbed with mouse Ig on BioGel bleeds	150	14.6 $\pm$ 1.9	(4)
4. Anti-mouse Ig	50	11.9 $\pm$ 3.5	(5)
5. Complement only	—	12.2 $\pm$ 2.6	(6)
6. No cells	—	0.2 $\pm$ 0.1	(4)

Expt 2: Positive selection

Cells transferred	Number	Spleen colonies	
7. Unfractionated CBA bone marrow	100,000	12.7 $\pm$ 3.1	(5)
8. 'Anti-Ia' negative cell	100,000	17.3 $\pm$ 4.2	(4)
9. 'Anti-Ia' positive cell	100,000	5.1 $\pm$ 2.7	(4)
10. No cells	—	0	

Condition as for Table 1. The 'anti-Ia antiserum' after exhaustive absorption with insolubilised Mlg stained  $\sim$  35% mouse spleen cells and  $\sim$  5% of mouse bone marrow cells at a dilution of 1:20 when developed with a fluoresceinated goat anti-rabbit Ig serum (Fab<sub>2</sub>).

contamination of the Ia<sup>+</sup> fraction with Ia<sup>-</sup> cells. The sorter was adjusted so that positive cells were excluded from the negative fraction but many cells which lacked Ia antigen were included in the Ia positive fraction.

Most of the studies of human 'Ia-like' antigens on human cells have used heteroantisera against Ia. Since these sera recognise only the non-polymorphic subunit<sup>7</sup> it is possible that only this portion of the molecule is present on the stem cells. They would thus escape detection by the alloantiserum used in the experiments reported in Table 1. To obviate this we have prepared a heterologous antiserum to mouse Ia antigens by injecting immunoprecipitates of Ia-alloanti-Ia complexes (precipitated with rabbit anti-mouse Ig) into rabbits. After four injections at 2-week intervals a serum was obtained which seems to contain anti-Ia antibodies. This serum, after exhaustive adsorption with insolubilised mouse Ig reacts with the majority of the Ig<sup>+</sup> cells of the spleen and bone marrow and also stains some Ig<sup>-</sup> cells from both organs. A detailed report of its properties will be presented elsewhere. Its effect on the mouse bone marrow cells which produce spleen colonies is shown in Table 2. In the first experiment the serum was added to CBA bone marrow cells with an effective source of complement and in the second FACS-separated C<sub>3</sub>H cells were injected: 19.6% of the cells are antigen positive compared with a background of 12.8% with normal rabbit serum (Fig. 1b). The results indicate that the cells identified by this heterologous antiserum to mouse Ia antigen are not CFU<sub>(s)</sub>. The serum does not kill CFU<sub>(s)</sub> and essentially all of the CFU are recovered in the 'Ia' negative fraction.

If these results can be generalised to man, it would seem that the cells recognised by the anti-'Ia' sera in human foetuses and in AML are not related to the most immature haematopoietic elements.

Ia antigens may only be expressed on progeny of these cells: cells which are already committed to either the lymphoid or myeloid cell lineages. Preliminary observations in man suggest that both Ia<sup>+</sup> and Ia<sup>-</sup> populations do contain myeloid precursors which form colonies when grown *in vitro* in semi-solid media (CFU<sub>(c)</sub>)<sup>20</sup> (G.J., X. Francis and M.F.G., unpublished). The basis for this heterogeneity has not been established but it seems possible that it is a consequence of the presence of cells of different degrees of maturity in the CFU<sub>(c)</sub> population.

Alternatively, it has been suggested that the expression of human Ia-like antigens is cell cycle dependent. CFU<sub>(s)</sub> in the mouse are not a mitotically active population. It is thus possible that



multipotential stem cells when driven into cell division might express Ia antigens but the resting cell normally present in the bone marrow seems to lack this antigen.

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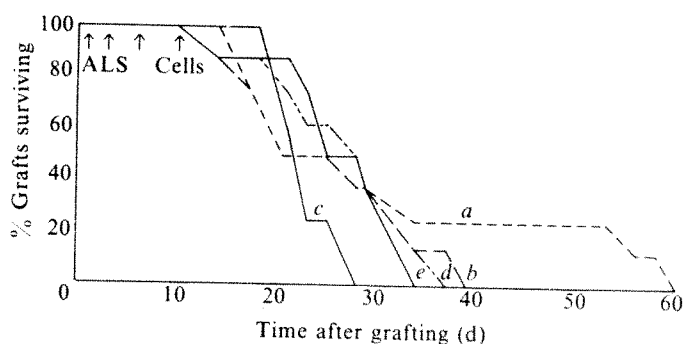
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1. Sachs, D. H. & Cone, J. A. *J. exp. Med.* **138**, 1289–1304 (1973).
2. David, C. S., Schreffler, D. C. & Frelinger, J. A. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2509–2514 (1973).
3. Hammerling, G. J., Mauve, G., Goldberg, E. & McDevitt, H. O. *Immunogenetics* **1**, 428–437 (1974).
4. Frelinger, J. A., Niederhuber, J. E., David, C. S. & Schreffler, D. C. *J. exp. Med.* **140**, 1273–1284 (1974).
5. Parish, C. R., Chilcott, A. B. & McKenzie, I. F. C. *Immunogenetics* **3**, 129–137 (1976).
6. Cullen, S. E., David, C. S., Schreffler, D. C. & Nathenson, S. G. *Proc. natn. Acad. Sci. U.S.A.* **7**, 648–652 (1974).
7. Barnstable, C. J. *et al. Cold Spring Harb. Symp. quant. Biol.* **41**, 443–456 (1977).
8. Schreffler, D. S. & David, C. S. *Adv. Immun.* **20**, 125–195 (1975).
9. Klein, J. & Hauptfeld, V. *Transplant. Rev.* **30**, 83–100 (1976).
10. Humphreys, R. E. *et al. J. exp. Med.* **144**, 98–112 (1976).
11. Snary, D., Barnstable, C. J., Bodmer, W. F., Goodfellow, P. M. & Crumpton, M. J. *Scand. J. Immun.* (in the press).
12. Billing, R. J., Safani, M. & Peterson, P. J. *Immun.* **117**, 1589–1593 (1976).
13. Welsh, K. I. & Turner, M. J. *Tiss. Antigens* **8**, 197–205 (1976).
14. Janossy, G. *et al. Br. J. Haematol.* (in the press).
15. Schlossman, S. F., Chess, L., Humphreys, R. E. & Strominger, J. L. *Proc. natn. Acad. Sci. USA* **73**, 1288–1292 (1976).
16. Janossy, G., Greaves, M. F., Sutherland, R., Durrant, J. & Lewis, C. *Leukaemia Res.* (in the press).
17. Winchester, R. J., Ross, G. D., Jarowski, C. I. & Wang, C. Y. *J. exp. Med.* (in the press).
18. Tilt, J. E. & McCulloch, E. A. *Radiat. Res.* **14**, 213–222 (1961).
19. Basch, R. S. & Kadish, J. L. *J. exp. Med.* **145**, 405–419 (1977).
20. Bradley, T. R. & Metcalf, D. *Aust. J. exp. biol. med. Sci.* **44**, 287–300 (1966).

## Absence of suppressor cells from rats bearing passively enhanced kidney allografts

LYMPHOCYTES which inhibit the immunological responses of other lymphocytes are known as suppressor cells, and such cells have been shown to mediate both specific and non-specific unresponsiveness in many experimental situations<sup>1–3</sup>. In the context of allotransplantation, Kilshaw *et al.*<sup>4,5</sup> have found that the highly strain-specific unresponsiveness to skin allografts induced in adult mice by pretreatment with donor strain tissue extract and *Bordetella pertussis* vaccine, followed by a short post-operative course of antilymphocyte serum (ALS), is at least in part mediated by thymus-dependent lymphocytes. Thus, transfer of  $25\text{--}100 \times 10^6$  spleen cells (optimally the lower dose<sup>5</sup>) from mice in the stable phase of unresponsiveness into ALS-treated syngeneic recipients established a long-lasting unresponsiveness to donor strain skin grafts in up to 50% of recipients. Because the nature of the mechanisms of immunological enhancement of kidney allografts in rats remain unclear<sup>6–8</sup> we have carried out cell transfer experiments similar to those of Kilshaw *et al.* in order to discover whether suppressor cells also play a part in mediating the stable phase of the enhanced state. Fabre and Morris<sup>9</sup> have previously failed to transfer unresponsiveness with spleen cells from rats with enhanced kidneys, but it is possible that transfer of cells to normal, syngeneic rats—as in their experiments—is an insufficiently sensitive method for detecting suppressor cells. Our results show that it is unlikely that suppressor cells or auto-anti-idiotypic antibodies are responsible for the steady state of enhancement. A more likely explanation involves the induction of unresponsiveness by the continuous exposure of the recipient to histocompatibility antigens in the absence of Ia-like antigens—the latter determinants being obligatory requirements for the activation of T cells ‘help-



**Fig. 1** Survival of AUG skin grafts in ALS-treated AS males following transfer of spleen and lymph node cells from normal AS female rats or female AS donors that had received passively enhanced F1 kidneys 5–9 months before (experiment 1). Cells were injected i.p. on day 10, ALS s.c. on days 1, 3 and 6 (arrows). ALS dose, 4 ml per rat per injection: 8 rats per group. *a*, Received  $175 \times 10^6$  cells from donors with enhanced kidneys. *b*, Received  $25 \times 10^6$  cells from donors with enhanced kidneys. *c*, Received  $175 \times 10^6$  cells from normal donors matched for age and sex. *d*, Received  $25 \times 10^6$  cells from normal donors matched for age and sex. *e*, ALS controls.

ing’ both potentially reactive B cells and T cells capable of differentiating into killer cells.

Passive enhancement of (AS $\times$ AUG)<sub>F1</sub> kidneys transplanted orthotopically to AS strain recipients was induced by injecting the recipients with 0.15–1.0 ml of AS-anti-AUG alloantiserum. As described previously<sup>6,7</sup>, this treatment leads to the production of a stable unresponsiveness, the majority of recipients continuing to live indefinitely with normal or slightly raised blood urea levels. The cell donors used in the three transfer experiments described here had been sustained by their grafts for 5–14 months, with a mean of 8 months.

The design of the transfer experiments was essentially the same as that used to demonstrate the presence of suppressor cells in the long-term unresponsive mice described by Kilshaw *et al.*<sup>4,5</sup>. Adult AS rats were grafted with AUG strain skin allografts on day 0; rabbit anti-rat lymphocyte serum (ALS), raised by two intravenous injections of approximately  $10^9$  AS lymph node cells, was administered subcutaneously to these animals either on days 2, 4 and 6 or on days 1, 3 and 6 after transplantation (for details see figure legends). On day 9 or 10, lymphoid cell suspensions were prepared from the AS rats bearing enhanced kidney allografts and injected in varying numbers into the prepared recipients (intraperitoneally in experiment 1 and intravenously in experiments 2 and 3).

Spleen and lymph node suspensions for transfer were prepared by shredding the tissues with fine forceps in RPMI 1640 medium to which 5% foetal calf serum had been added. The coarse suspension was then sieved and clumps were removed by rapid passage through a cotton wool filter or by allowing the clumps to settle. The resulting single cell suspensions were centrifuged for 10 min at 1,200 r.p.m., resuspended, counted and assessed for viability.

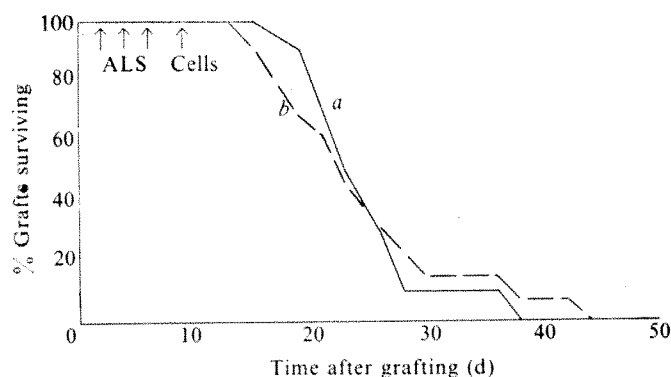
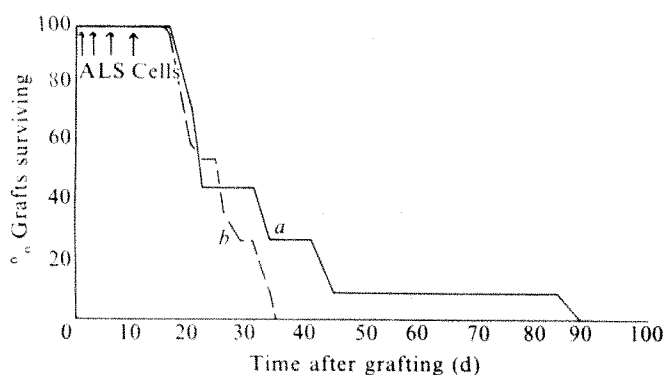
The results were clear-cut. In experiment 1, neither high nor low doses of mixed spleen and lymph node cells transferred unresponsiveness, although 2/8 of the grafts in the high dose group exceeded the median survival time of the control grafts by 20–25 d (Fig. 1). High doses of normal cells somewhat curtailed graft survival, as might be expected. In the second experiment (Fig. 2) only 1/11 grafts in the group receiving spleen cells showed prolonged but limited survival beyond that of the controls; in the third (Fig. 3) there was no difference whatever between experimental and control groups. According to the Mann–Whitney test there were no statistically significant differences between the experimental and control group of experiments 1 and

2 ( $P = >0.05$ ). It must be concluded that splenic suppressor cells do not have a significant role in mediating the stable phase of the enhanced state, unless we have been singularly unfortunate in our choice of the number of cells transferred. The lower dose (Fig. 1) corresponds to that giving optimal results in mice<sup>5</sup>, and the higher dose was intended to take into account the body weight differences between mice and rats.

These experiments also provide evidence against the participation of auto-anti-idiotypic immunity in the 'steady state' of enhanced kidney allografts. Binz *et al.*<sup>10</sup> have shown that B- and T-cell recognition structures have idiotypes in common, and that anti-idiotypic antibodies will combine with subpopulations of T and B cells which react with the same transplantation antigens<sup>11</sup>. Because auto-anti-idiotypic antibody production can be provoked in Lewis rats either directly by immunisation with purified natural antibody<sup>12</sup> or with specifically reactive autologous T cells<sup>13</sup>, or indirectly by allogeneic tissue<sup>14</sup>, kidney allograft survival could be due to the development of auto-anti-idiotypic immunity<sup>15</sup>. Whilst auto-anti-idiotypic immunity can undoubtedly be induced in both mice and rats, and can cause a specific unresponsiveness<sup>16</sup>, it has yet to be shown that this is the major mechanism which protects kidney allografts during the steady state of enhancement. If auto-anti-idiotypic immunity were involved it should be possible to transfer the unresponsiveness adoptively with lymphoid cells, but our experiments show that this does not happen. Other evidence against the auto-anti-idiotypic hypothesis is provided by the finding that kidney allograft protection cannot be achieved convincingly by actively immunising AS rats with purified AS-anti-AUG antibody derived from enhancing sera<sup>7</sup>, despite the fact that in this strain combination enhancement can be brought about with relatively small doses of antiserum; and by the observation that passive immunisation of Lewis rats with Lewis anti-(Lewis-anti-BN) antibody only occasionally prolongs the survival of (Lewis × BN)<sub>F</sub><sub>1</sub> kidney allografts<sup>18</sup>.

If neither suppressor cells nor anti-idiotypic antibodies are primarily involved in mediating the stable phase of the enhanced state, what other mechanism is likely to be operative? Cantor and Boyse<sup>17</sup> have shown that T lymphocytes can be divided into at least two populations, one responding to Ia-like antigens and the other maturing into killer T lymphocytes with specificity for the classical histocompatibility (H) antigens. The Ia-responsive population of T cells provides help both for the B cell lineage, which synthesises IgG antibody against the thymus-dependent MHC antigens<sup>18,19</sup>, and the T-cell clones, which differentiate into

**Fig. 2** Survival of AUG skin grafts in ALS-treated AS females following transfer of spleen cells from female AS donors that had received their passively enhanced F1 kidneys 5–8 months before (experiment 2). Cells injected i.v. on day 10, ALS s.c. on days 1, 3 and 6 (arrows). ALS dose, 1.0 ml per 50 g body-weight per injection: 11 rats per group. *a*, Received  $200 \times 10^6$  cells from donors with enhanced kidneys. *b*, ALS controls.



**Fig. 3** Survival of AUG skin grafts in ALS-treated AS males following transfer of spleen cells from male AS donors that had received their passively enhanced F1 kidneys 9–14 months before (experiment 3). Cells injected i.v. on day 9, ALS s.c. on days 2, 4 and 6 (arrows). ALS dose, 1.0 ml per 40 g body weight per injection. *a*, Received  $200 \times 10^6$  cells from donors with enhanced kidneys (11 rats). *b*, ALS controls (13 rats).

killer T lymphocytes with specificity for H-type antigens<sup>20,21</sup>. Because kidneys contain little or no Ia-type antigen<sup>22</sup>, and long-surviving allografts must be devoid of donor-type passenger leukocytes, during the steady state of enhancement the recipient is exposed to a continuous presence of H-type antigen in the absence of stimulation by Ia. We postulate that this exposure not only fails to provoke immunity but also induces cumulatively a specific non-reactivity of the T and B precursor cells which, if provided with 'help', would normally have differentiated into T killers and antibody producers, respectively, with specificity for H-type antigens.

In keeping with this hypothesis is the finding that pretreatment of rats with allogeneic platelets leads to suppression rather than activation of humoral immunity<sup>23</sup>, for platelets possess H antigens but lack Ia. Consistent depression of killer T lymphocyte generation after platelet injection was not observed, but only a limited number of dose schedules have so far been examined. Because prolonged kidney allograft survival has been observed after pretreatment of recipients with platelets<sup>24</sup>, and Wagner and Nossal<sup>21</sup> have shown that cellular immunity generated *in vitro* can be specifically suppressed by allogeneic membranes provided that high doses are added to the cultures, the question of dosage effects *in vivo* clearly needs further examination.

It has been suggested that induction of enhancement is due to the opsonisation of antigen-reactive cells by antigen-antibody complexes and their subsequent removal through phagocytosis<sup>25</sup>. The maintenance of the steady state by this mechanism would require the continuous production of anti-donor antibody. Because the amount of antibody required for maintenance (as opposed to induction) may be quite small our transfer experiments do not necessarily exclude this possibility.

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1. Gershon, R. K. *Transpl. Rev.* 26, 170-185 (1975).
2. Tada, T., Taniguchi, M. & Takemori, T. *Transpl. Rev.* 26, 106-129 (1975).
3. R. W. Dresser (ed.) *Immunological Tolerance, Br. med. Bull.* 32, 99-191 (1976).
4. Kilshaw, P. J., Brent, L. & Pinto, M. *Nature* 255, 489-491 (1975).
5. Kilshaw, P. J. & Brent, L. *Transpl. Proc.* 9, 717-719 (1977).
6. French, M. E. & Batchelor, J. R. *Transpl. Rev.* 13, 115-141 (1972).
7. Batchelor, J. R. & Welsh, K. I. *Br. med. Bull.* 32, 113-117 (1976).
8. Carpenter, C. B., d'Apice, A. J. B. & Abbas, A. K. *Adv. Immun.* 22, 1-65 (1976).
9. Fabre, J. W. & Morris, P. J. *Transplantation* 14, 634-640 (1972).
10. Binz, H., Lindenmann, J. & Wigzell, H. *J. exp. Med.* 140, 731-741 (1974).
11. Binz, H. & Wigzell, H. *J. exp. Med.* 142, 197-211 (1975).
12. Binz, H. & Wigzell, H. *Nature* 262, 294-295 (1976).
13. Binz, H. & Wigzell, H. *Nature* 264, 778-780 (1976).
14. McKearn, T. J., Stuart, F. P. & Fitch, F. W. *J. Immun.* 113, 1876-1882 (1974).
15. Stuart, F. P., Scollard, D. M., McKearn, T. J. & Fitch, F. W. *Transplantation* 22, 455-466 (1976).
16. Anderson, L. C., Binz, H. & Wigzell, H. *Nature* 264, 778-780 (1976).
17. Cantor, H. & Boyse, E. A. *J. exp. Med.* 141, 1376-1389; 1390-1399 (1975).
18. Klein, J., Livnat, S., Hauptfeld, V., Jerábek, L. & Weissman, I. *Eur. J. Immun.* 4, 41-44 (1974).
19. Rolstad, B., Williams, A. F. & Ford, W. L. *Transplantation* 17, 416-423 (1974).
20. Eijssvoegel, V. P. et al. *Transpl. Proc.* 5, 415-420 (1973).
21. Wagner, H. & Nossal, G. J. V. *Transpl. Rev.* 17, 3-36 (1974).
22. Hammerling, G. J. *Transpl. Rev.* 30, 64-82 (1976).
23. Welsh, K. I., Burgos, H. & Batchelor, J. R. *Eur. J. Immun.* 7, 267-272 (1977).
24. Batchelor, J. R., Welsh, K. I. & Burgos, H. *Transpl. Proc.* 9, 931-936 (1977).
25. Hutchinson, I. V. & Zola, H. *Transpl. Proc.* 9, 961-963 (1977).

## HLA restriction of cell-mediated lysis of influenza virus-infected human cells

MURINE T lymphocytes that mediate the lysis of virus-infected cells show specificity both for the viral cell surface antigens and for the H-2K or D antigens of the major histocompatibility complex<sup>1-8</sup>. The cytotoxic T lymphocytes and the target cell must share H-2K or D products. The experiments reported here demonstrate that there is a similar requirement for partial HLA identity between human cytotoxic lymphocytes and influenza virus-infected target cells.

The viruses used in this study were influenza type A/X31 (A<sub>2</sub>/Hong Kong/68 × A<sub>0</sub>/PR8 (H3N2) and type B/Hong Kong (B/Hong Kong/1/73). H-2 compatibility has been shown to be required for T-lymphocyte-mediated lysis of influenza virus-infected target cells in mice<sup>9-11</sup>. Although there was no cross reaction between type A and B influenza viruses, type A viruses with serologically distinct surface proteins were found to cross react both at the level of secondary induction of cytotoxic cells and in the recognition of infected target cells<sup>7-9</sup>. Consequently, we anticipated that deliberate immunisation of the human volunteers studied would not be necessary, because nearly

all adults have been infected with some influenza viruses of the A group. Similarly, most would have been exposed to viruses of the B type. The individuals studied were therefore asked only to give venous blood samples.

Influenza viruses grown on chick allantoic membranes were used to infect peripheral blood lymphocytes, prepared from heparinised blood by centrifugation on Ficoll-Hypaque. These cells were exposed to virus and after a further 4-h incubation they could be lysed by rabbit antibody to type A influenza virus and complement and therefore expressed viral antigens on their surface. Such infected cells served as stimulator cells for *in vitro* sensitisation or target cells for cell-mediated lysis (CML) (ref. 10). A similarly infected lymphoblastoid cell line (PGF) was also a good target.

Cytotoxic cells could be generated by sensitising peripheral WBC with influenza virus-infected autologous cells, *in vitro*, for 5-8 d at 37 °C. Cell-mediated lysis was then assayed for 5 h on a panel of freshly prepared peripheral blood lymphocytes which were virus-infected and <sup>51</sup>Cr-labelled. These conditions were found to be optimal in preliminary experiments with killer target cell ratios of 40:1 or 50:1. After sensitisation with influenza virus type A/X31 or B/Hong Kong, peripheral blood lymphocytes from J.D. and F.W. killed infected autologous cells, and cytotoxicity was specific for the immunising virus type A or B (Table 1). C.W. cells could be sensitised to A/X31 but not to B/Hong Kong which may indicate that C.W. had not been previously exposed to the B/Hong Kong virus. Otherwise it was apparent that previous exposure to virus *in vivo* had resulted in immunological priming or, less likely, that the cytotoxicity generated *in vitro* represented a primary immune response. Freshly prepared peripheral blood lymphocytes showed no cytotoxic activity towards infected cells and therefore a period of sensitisation *in vitro* was essential. The finding that sensitised J.D. and F.W. cells showed specificity for the virus used for sensitisation only, also confirmed this observation.

The infected target cells used in the cytotoxic assay had been incubated long enough to express viral antigens at the cell surface. Zweerink and Askonas (unpublished data) have shown in the mouse, that this was required before influenza-infected cells could be lysed by cytotoxic T

Table 1 Human cytotoxic lymphocytes show specificity for influenza virus type A or B

Cell donor	Sensitised to influenza type	% <sup>51</sup> Cr release* from target cells infected with		
		A/X31	B/Hong Kong	Uninfected
J.D.	A/X31	36.0	20.2	18.2
	B/Hong Kong	27.3	37.0	18.4
Medium control		21.0	19.5	15.5
F.W.	A/X31	35.5	21.3	15.5
	B/Hong Kong	20.5	35.4	15.5
Medium control		16.0	17.7	15.1
C.W.	A/X31	52.8	13.1	10.3
	B/Hong Kong	29.4	19.4	11.3
Medium control		16.7	9.9	10.5

\* % <sup>51</sup>Cr release = (counts released by target cells/counts released by 2.5% Triton X-100) × 100. <sup>51</sup>Cr-release assay was carried out in triplicate in microtitre II plates<sup>10</sup> using 10<sup>4</sup> virus-infected <sup>51</sup>Cr-labelled peripheral blood lymphocytes and 5 × 10<sup>5</sup> immune cells. Incubation was for 5 h.

Target cells were prepared on the day of the CML assay by infecting peripheral blood lymphocytes with influenza virus type A/X31 or B/Hong Kong (see below). Target cells were radiolabelled by the addition of 100 μCi <sup>51</sup>Cr during the period of infection (90 min), excess <sup>51</sup>Cr was removed by washing cells three times and incubation was continued for 4 h at 10<sup>6</sup> cells ml<sup>-1</sup> in RPMI 1640 medium-10% FCS. J.D. and F.W. were each tested against autologous target cells, and C.W. against HLA compatible target cells. Human peripheral blood lymphocytes were prepared from heparinised venous blood by centrifugation on Ficoll-Hypaque. The cells at the interface were collected and the red blood cells lysed by a 20-min incubation with Tris-buffered 0.75% ammonium chloride at 37 °C. The cells were then washed three times in RPMI 1640 medium (containing 25 mM Hepes buffer, 200 mM glutamine, penicillin and streptomycin). An aliquot of 3 × 10<sup>6</sup> lymphocytes was incubated in 0.2 ml with 100 HA units of influenza virus (A/X31 or B/Hong Kong) at 37 °C for 90 min. After a single wash the cells were incubated for 4 h at 37 °C in 3 ml of RPMI 1640 medium containing 10% foetal calf serum (FCS). They were then washed three times and added to the remaining uninfected peripheral blood mononuclear cells at a ratio of 1:20. The cells were incubated at 10<sup>6</sup> ml<sup>-1</sup> in 4-ml volumes in Bijou bottles (Sterilin) in RPMI 1640-10% FCS for 5-8 d at 37 °C in 5% CO<sub>2</sub> air.



Table 2 Human cytotoxic lymphocytes show specificity for HLA

Cytotoxic cell	% Lysis of influenza A/X31-infected target cells*		
	J.D. (3, 7, W2/2, 7, W2)	F.P. (3, 7, W2/1, 8, -)	F.W. (1, 8, -/1, 8, W3)
C.W. (9, 7, W2/9, 7, W1)	43	30	5
F.W. (1, 8, -/1, 8, W3)	6	16	23

C.W. and F.W. peripheral blood lymphocytes were sensitised to their own lymphocytes infected with influenza type A/X31, and were then tested for cytotoxicity against the influenza type A/X31-infected target cells shown. The HLA types are shown for each individual in the sequence A, B, D/A, B, D.

$$*\% \text{ Lysis} = \frac{^{51}\text{Cr release} - \text{medium control } ^{51}\text{Cr release}}{(^{51}\text{Cr release by Triton X 100}) - (\text{medium control } ^{51}\text{Cr release})} \times 100$$

lymphocytes. Similarly, sensitised human cells failed to lyse autologous cells exposed to ultraviolet-irradiated virus<sup>9</sup>.

Having established that cytotoxic cells show virus specificity the possibility that their activity is HLA-restricted was explored. HLA A, B and C typing was carried out by the standard Terasaki-NIH microcytotoxicity technique. All the individuals studied had also been typed as normal panel blood donors, for B cell (Ia) alloantigens by sera exchanged in the UK regional workshops<sup>11</sup> and Seventh

International workshop. The D locus types thus assigned are those of the UK groups which have been found to correlate with the D specificities defined by mixed lymphocyte typing<sup>11</sup>. The DWI-3 terminology of the Sixth Workshop<sup>12</sup> is therefore retained.

Effector cells specific for influenza type A/X31 were generated from C.W. (HLA 9,7,W2/9,7,W1) and F.W. (HLA 1,8,W3/1,8,-) and these were tested against virus-infected target cells prepared from C.W., F.W. and F.P. (HLA 3,7,W2/1,8,-) (Table 2). Cytotoxic cells from each individual killed the autologous or partially HLA-matched cells but not the HLA-incompatible cells. Each target cell population tested was lysed by at least one effector, which excluded the possibility that a target might be inadequately infected.

This HLA restriction was explored further using C.W. as the effector cell. C.W. lymphocytes sensitised to A/X31 virus were tested against A/X31 virus-infected peripheral blood lymphocytes from 15 individuals, and one lymphoblastoid cell line (P.G.F.). Figure 1 shows the lysis and its correlation with the degree of HLA compatibility between the target cells and C.W. Infected lymphocytes which shared HLA B7 with C.W. were killed—mean percentage lysis  $38.1 \pm 1.9$  s.e.m. Targets that lacked HLA B7 showed minimal lysis; mean  $9.0 \pm 2.6$  s.e.m. This difference was highly significant: (Students'  $t$  10.8  $P < 0.0001$ ). When the target cells shared HLA A9 (W24) or the D locus products DW1 or DW2 only, lysis was not observed.

It is therefore apparent that sharing of HLA B7 with the effector cell was sufficient to allow lysis, whereas sharing of the A or D locus products with C.W. was insufficient. Because it was not feasible to test each target-cell preparation with its autologous sensitised cells, with the exception of F.W. (Table 2), it is theoretically possible that only the HLA B7 positive cells were adequately infected. In previous experiments, however, sensitised cells from eight individuals who were HLA B7 negative lysed autologous influenza A/X31 infected target cells, so that the infection of cells is reproducible.

These findings therefore extend to the human the original observations of Zinkernagel and Doherty<sup>13</sup> that, in the mouse, lymphocyte-mediated lysis of virus-infected cells is restricted by the major histocompatibility complex. This phenomenon is associated with the H-2K and D products which are chemically homologous to HLA A and B antigens<sup>14</sup>. It is therefore appropriate that the restriction involves HLA B in man and that HLA D, which is probably equivalent to the H2 I region, was not involved. It is not clear why in the one pair tested, sharing of the A locus antigen A9 (W24), did not allow lysis. Goulmy *et al.*<sup>15</sup> have found, however, that a human (female) cytotoxic cell population sensitised to the H-Y antigen killed targets that shared an HLA A antigen. It seems, therefore, that HLA B and A are functionally equivalent to H-2K and D. Failure to observe lysis when HLA A9 (W24) was shared might therefore be an effect specific to influenza antigen. Alternatively, the A9 (W24) type might include sub-

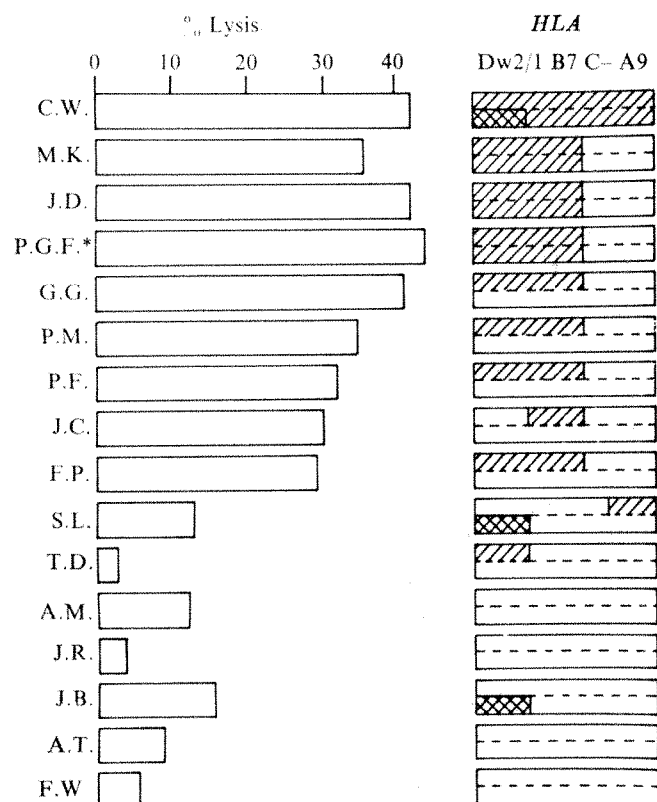


Fig. 1 HLA restriction of cell-mediated lysis of influenza type A/X31-infected cells. C.W. peripheral blood lymphocytes were sensitised to autologous lymphocytes infected with influenza type A/X31, and tested for cytotoxicity against the target cells shown. Results shown as percentage lysis (see legend to Table 2) in the left-hand side of the figure. The degree of HLA compatibility is shown on the right-hand side of the diagram. Sharing of HLA DW2, or B7, C-, A9 is represented by diagonal hatching either as one haplotype (top half only) or two haplotype compatibility (both halves). Sharing of HLA DW1 is shown by cross-hatching. The HLA A, B, D types of the target cells were C.W.: (autologous target), 9(W24), 7, W2/9 (W24), 7, W1; M.K.: 3, 7, W2/3, 7, W2; J.D.: 3, 7, W2/2, 7W2; \*P.G.F.: 3, 7, W2/3, 7, W2; G.G.: 2, 3, 7, W14, -, W2; F.P.: 3, 7, W2/1, 8, -; S.L.: 9(W24), W30, 13, W15, W1, -; T.D.: 2, 2, 12, W40, 2, -; A.M.: 2, 21, -/W32, W40, -; J.R.: 2, 2, 12, 27, -, -; J.B.: W30, W32, W15, -, W3, W1; A.T.: 2, 11, W40, W22, -, -; F.W.: 1, 8, W3/1, 8, -.

\*P.G.F. was a lymphoblastoid cell line, donated by Professor W. F. Bodmer.

groups that, although serologically related, might not behave as an identical pair in target-cell recognition. This would be analogous to the failure of H-2<sup>b</sup> mouse cytotoxic cells to recognise target cells that share the mutant H-2<sup>ba</sup> K antigen<sup>16</sup>. Indeed, if such mutants are common in the human population, apparent HLA homozygotes that are really wild × mutant HLA heterozygotes would lyse a larger selection of target cells than true homozygotes.

The HLA types of the individuals used as cytotoxic cell donors show linkage disequilibrium. The haplotypes A1-B8-DW3 and B7-DW2 occur in Caucasian populations at frequencies that are much greater than would be expected from the gene frequencies of the individual antigens<sup>17</sup>. This means that, if the recognition of target cells involves genes closely linked to HLA-B, these are likely to be the same when killer and target cells share an HLA haplotype. This is another possible explanation for the lack of killing when HLA A9 (W24), which is not in linkage disequilibrium with B7, was shared.

This finding that there is HLA restriction of cell-mediated lysis of influenza virus-infected cells is in accord with data in the mouse showing that for influenza<sup>3-8</sup> and other viruses<sup>1-4</sup> there is H2 restriction. It contrasts with reports that human cytotoxic T lymphocytes showed no HLA restriction in the lysis of Epstein-Barr virus<sup>17</sup> or measles virus<sup>18</sup>-infected cell lines.

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1. Doherty, P. C., Blanden, R. V. & Zinkernagel, R. M. *Transplant. Rev.* **29**, 89-124 (1976).
2. Koszinowski, U. & Ertl, H. *Nature* **255**, 552-554 (1975).
3. Blanden, R. V. *et al.* *Nature* **254**, 269-270 (1975).
4. Doherty, P. C. & Zinkernagel, R. M. *Immunology* **31**, 27-32 (1976).
5. Zweerink, H. J., Courtneidge, S. A., Skehel, J. J., Crumpton, M. J. & Askonas, B. A. *Nature* **267**, 354-356 (1977).
6. Yap, K. L. & Ada, G. L. *Immunology* **32**, 151-160 (1977).
7. Effros, R. B., Doherty, P. C., Gerhard, W. & Bennink, J. J. *exp. Med.* **145**, 557-568 (1977).
8. Doherty, P. C., Effros, R. B. & Bennink, J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1209-1213 (1977).
9. Zweerink, H. J., Askonas, B. A., Millican, D., Courtneidge, S. A. & Skehel, J. J. *Eur. J. Immun.* (in the press).
10. Simpson, E., Gordon, R., Taylor, M., Mertin, J. & Chandler, P. *Eur. J. Immun.* **5**, 451-455 (1975).
11. Bodmer, J. *et al.* *Tissue Antigens* **8**, 359-371 (1976).
12. *Histocompatibility Testing 1975*. (ed. F. Kissmeyer-Nielsen) (Munksgaard, Copenhagen, 1976).
13. Zinkernagel, R. M. & Doherty, P. C. *Nature* **251**, 547-548 (1974).
14. Hood, L. in *The Role of Products of the Histocompatibility Gene Complex in human responses*. (ed. D. H. Katz & B. Benacerraf) 703-705 (Academic, London, 1976).
15. Goulmy, E., Termijtelen, A., Bradley, B. A. & Van Rood, J. J. *Nature* **266**, 544-545 (1977).
16. Zinkernagel, R. M. *J. exp. Med.* **143**, 437-443 (1976).
17. Jondal, M., Svedmyr, E., Klein, E. & Singh, S. *Nature* **255**, 405-407 (1975).
18. Ewan, P. & Lachmann, P. *Clin. exp. Immun.* (in the press).

## Human cell-mediated cytotoxicity against modified target cells is restricted by HLA

T-CELL-mediated lysis of virus-infected target cells in mice is restricted by the H-2D and H-2K antigens of the major histocompatibility complex (MHC) in the sense that the cytotoxic T cells are only active against virus-infected target cells sharing H-2D and/or H-2K antigens with the animal in which the killer cells have been raised<sup>1</sup>. These findings led to a better understanding of the way in which the MHC regulates certain functions of the immune system<sup>2</sup>, and they have been amply confirmed by further studies in inbred strains of mice and guinea pig which have indicated that restriction by antigens of the MHC is a general phenomenon in the T-cell-mediated immune response<sup>3</sup> in these species. Evidence for MHC restriction of the immune response in man, however, was lacking until it was shown that a female patient who had rejected a bone marrow graft from her HLA-identical brother, had cytotoxic cells in her blood, and that the activity of these cells was restricted to male target cells which had the HLA-A2 antigen in common with herself<sup>4</sup>. We show here in a more general way, that the cell-mediated cytotoxicity which can be raised in humans against 'altered self' is restricted by the HLA-A, B and C antigens.

It has been shown that peripheral lymphocytes from subjects who have been sensitised by skin painting with DNCB respond with proliferation when cocultured *in vitro* with autologous irradiated and dinitrofluorobenzene (DNFB)-conjugated lymphocytes<sup>5</sup>. We have investigated the generation of cytotoxic cells in such cultures. Individuals were sensitised to DNCB following their informed consent, as a part of a clinical study of the immune response in a group of patients suffering from various allergic disorders, primarily asthma bronchiale. Within this group, subjects with minor disease activity gave permission to use some of their blood samples for a study not directly related to their disorder. A total of 27 subjects were examined. All showed contact sensitivity against DNCB. This hypersensitivity followed either the application of 2,000 µg DNCB dissolved in 50 µl acetone on the volar surface of each forearm, or subcutaneous injection of 25 × 10<sup>6</sup> DNFB-treated autologous lymphocytes. All subjects were investigated 2-5 weeks after the sensitisation by the method described in the legend to Table 1. Briefly, mononuclear blood cells were mixed with an equal amount of DNFB-treated autologous mononuclear cells and cultured for 8 d *in vitro*. The cytotoxic capacity of these *in vitro* restimulated cells was then investigated on <sup>51</sup>Cr-labelled, DNFB-treated autologous PHA-stimulated lymphocytes, revealing that 14 of the 27 individuals produced cytotoxic cells against autologous DNFB-coupled target cells. Eight were strongly positive and of these, one could not be retested, and two had lost most of the reactivity when retested 2-3 weeks after the first investigation. The results of retesting the remaining five individuals against selected panels of unrelated allogeneic lymphocytes are given in Table 1. The panels were selected according to the HLA types of the sensitised individuals and represented various degrees of HLA disparity. Freshly explanted lymphocytes from the sensitised subjects had no direct cytotoxic activity, and we have not succeeded in generating DNFB-directed cytotoxic cells *in vitro* with lymphocytes from non-immunised subjects.

It is obvious that cytotoxic activity is directed only against DNFB-coupled target cells. Of these, only autologous targets or targets sharing one or more HLA-A,B antigens with the cytotoxic effector cell were lysed, whereas there was no or (in two cases) only weak activity against totally HLA-A,B different target cells. These results show

Table 1 DNFB-directed CML against autologous and allogeneic DNFB-treated and untreated target cells

Sens. donor KR*: HLA-A2, B7, 40, Cw3			Sens. donor VH*: HLA-A1, 2, B5, 17		
Targets:			Targets:		
	(% Specific <sup>51</sup> Cr-release) DNFB-treated target	Untreated target		(% Specific <sup>51</sup> Cr-release) DNFB-treated target	Untreated target
Autologous	66	2	Autologous	35	0
A2, B7, 40, Cw3	54	4	A1, 2, B5, 17	35	2
A2, B8, 17	61	1	A1, 2, B44, w38, Cw4	36	3
A1, 3, B7, 40, Cw3	15	1	A3, 9, B5, 17, Cw2	7	-1
A1, 3, B8, 37	5	1	Aw19, B12	2	-2
A25, 29, B44	3	1	A25, 26, B18, w21, Cw4	2	-1
Sens. donor JR*: HLA-A2, B44, 15, Cw3			Sens. donor MC†: HLA-A1, 25, B17, 18		
Targets:			Targets:		
Autologous	50	3	Autologous	24	1
A2, B44, 15, Cw3	63	-1	A1, B17	19	1
A2, B44, 15, Cw1	46	0	A1, 10, B8	6	0
A2, B8, 17	55	0	A2, w24, B18, 44	19	T.F.
A1, 3, B44, 15, Cw3	3	1	A2, w32, B40, 44, Cw2	13	1
A3, 29, B44, 15, Cw3	3	-2	A2, 11, B15, w41, Cw3	1	2
A11, Bw21, 35, Cw4	4	3	SRBC-rosetted effector cells against autologous target (see text)	34	1
Sens. donor OT†: HLA-Aw24, 26, B27, Cw2					
Targets:					
Autologous	34	3			
Aw24, 25, B27, 40, Cw1, 3	13	2			
Aw24, 26, Bw16	13	5			
A2, Bw35, 40, Cw3	9	2			

Mononuclear cells were isolated by Ficoll-hypaque flotation from defibrinated venous blood. Responder cells from the sensitised donors were resuspended to a concentration of  $1.5 \times 10^6$  cells  $\text{ml}^{-1}$  in RPMI-1640 supplemented with antibiotics, glutamine and 15% pooled heat-inactivated human serum ('Ned. H'). Stimulator cells were resuspended in RPMI-1640 ( $\approx 5 \times 10^6$  cells  $\text{ml}^{-1}$ ) and to each ml of suspension was added 0.1  $\mu\text{l}$  of a 2% solution of DNFB in acetone, followed by incubation at room temperature in the dark for 30 min. The DNFB-treated cells were washed twice, resuspended in Med. H at a concentration of  $1.5 \times 10^6$  cells  $\text{ml}^{-1}$  and irradiated with 2,300 rad. Equal amounts of responder and stimulator cell suspensions were mixed and incubated in flat-bottomed tubes in 5-ml portions for 8 d at 37 °C in a humidified atmosphere with 5%  $\text{CO}_2$ . The portions were then pooled, the cells washed once, and resuspended in fresh Med. H at a concentration of  $5 \times 10^6$  Trypan-blue excluding cells per ml (effector cells). Target cells were prepared by incubating  $2 \times 10^6$  phytohaemagglutinin (PHA)-stimulated mononuclear cells (1  $\mu\text{l}$  PHA-P (Wellcome) per ml suspension of  $1 \times 10^6$  cells  $\text{ml}^{-1}$  in Med. H incubated for 3 d) with 250  $\mu\text{Ci}$   $\text{Na}_2^{51}\text{CrO}_4$  in 0.5 ml RPMI for 45 min at 37 °C. Some of the target cells were coupled with DNFB as described above, but five times as much DNFB was used per ml of cell suspension. The washed target cells were resuspended at a concentration of  $1 \times 10^5$  cells  $\text{ml}^{-1}$  in Med. H. 200  $\mu\text{l}$  of the effector cell suspension and 100  $\mu\text{l}$  of the target cell suspension were mixed in roundbottomed microtest tubes and incubated for 5 h at 37 °C. The test tubes were centrifuged and radioactivity in the supernatant and cell pellet was counted in a gammacounter. Target cells were incubated in parallel in medium alone and the  $^{51}\text{Cr}$  releases were calculated as percentage of  $^{51}\text{Cr}$  released relative to the amount incorporated in the target cells at the start of the experiment. Specific  $^{51}\text{Cr}$ -release was expressed as (release in mixtures with effector cells) - (release in medium alone). The releases in medium (control) were between 7% and 15%. Experiments were carried out in duplicate or triplicate, and s.e.m.s. were between 1 and 3%. A reaction was considered positive when the specific release exceeded 5%.

\* Donors KR, JR and VH were sensitised by injection of autologous DNFB-treated cells.

† Donors OT and MC were sensitised by the application of DNCB on the skin.

TF, Technical failure.

Antigens in common between effector and target cells and italicised. Two cases of target cell killing not restricted by HLA-A, B, C are also italicised.

that the phenomenon of MHC-restricted cell-mediated cytotoxicity also applies to man. We have no unequivocal proof that the cytotoxicity is mediated by T cells. However, more than 90% of the viable cells in the washed effector cell suspension formed rosettes with sheep red blood cells (SRBC). In donor MC an SRBC-rosetted effector cell suspension was separated by Ficoll-Hypaque density flotation. Too few cells were found in the interphase (non-rosetted cells) to be tested for cytotoxic activity. The sedimented SRBC-rosetted cells were collected and after lysis of the SRBC by fresh human AB serum, the cells were tested for cytotoxic activity. These cells had strong DNFB-directed cytotoxic activity (Table 1).

In the three cases where the effector cell donor was HLA-A2, most of the reactivity could be explained by preferential restriction by A2 alone. In fact, the experiments involving this antigen gave the highest specific releases. This might suggest that there is a hierarchy of antigenicity among the antigenic determinants produced by DNFB treatment, in the sense that one HLA antigen conjunction, when available, is preferred to the other possible conjunctions. Preferential restriction by only some of the MHC antigens available has been seen in several mice<sup>6-8</sup>, and might be

caused either by properties of the MHC antigens themselves or by immune response genes. Whereas the first possibility seems most likely in the present case (particularly the A2 antigen may have a unique role in cytotoxicity), evidence has been presented which evokes Ir genes in the mice systems<sup>6,7</sup>. Only some of the sensitised donors generated DNFB-directed cytotoxicity detectable by our *in vitro* system. The successful cases could be fortuitous results of a complex multifactorial interaction, but it is possible that a major factor of genetically determined immune responsiveness may have a role. Further studies are needed to clarify these questions, which have obvious implications for our understanding of the maintenance of MHC polymorphism.

The two cases of non HLA-A, B, C restricted target cell killing (if not technical artefacts) could be explained by (1) cytotoxicity exclusively directed against DNFB; (2) DNFB treatment may make target cells liable to killing by cytotoxic effector cells of any specificity; (3) cytotoxicity could be directed against DNFB-'modified' common cell-surface components other than the known HLA-A,B,C antigens; and (4) cross reactions between DNFB-'modified' HLA-A, B, C antigens which are not necessarily revealed by



the known serological cross reactions between HLA antigens. The first two possibilities seem unlikely in view of the many negative reactions with DNFB-treated target cells shown in Table 1.

The DNFB-directed cytotoxic activity could be induced *in vitro* only in lymphocytes from sensitised donors, whereas alloaggressive cytotoxicity is easily induced after primary sensitisation *in vitro*<sup>9</sup>. This presumably reflects the phenomenon emphasised by Simonsen<sup>10</sup>, that the number of antigen-sensitive cells primarily reactive against allotypic MHC-antigens in an unsensitised organism is much greater than that of cells primarily reactive against other antigens.

The main purpose of the present study was to investigate whether the phenomenon of MHC-restricted cell-mediated cytotoxicity applies also in man. Having shown that this is the case, work is now in progress to help resolve some of the new questions raised. Moreover, it seems likely that studies of contact hypersensitivity diseases with modifications of the present technique could elucidate the pathogenesis in these disorders.

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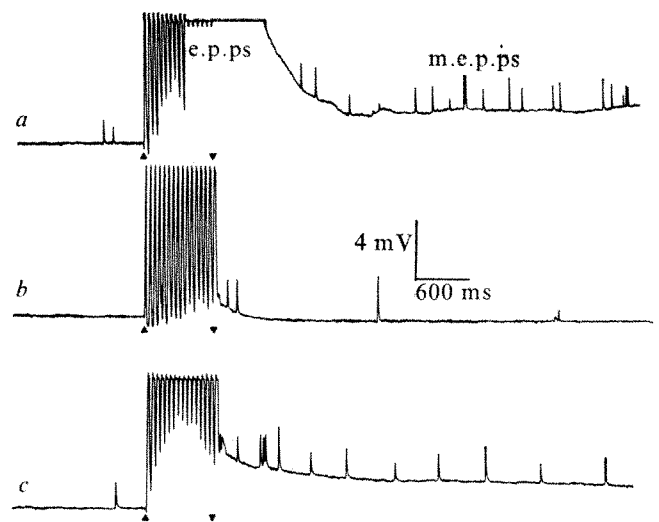
1. Zinkernagel, R. M. & Doherty, P. R. *Nature* **248**, 701–702 (1974).
2. Doherty, P. C. & Zinkernagel, R. M. *Lancet* **i**, 1406–1409 (1975).
3. Miller, J. A. F. P., Vadas, M. A., Whitelaw, A. & Gamble, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2486–2490 (1976).
4. Goulmy, E., Termijtlen, A., Bradley, B. A. & van Rood, J. J. *Nature* **266**, 544–545 (1977).
5. Sæberg, B. & Andersen, V. *Clin. exp. Immun.* **25**, 490–492 (1976).
6. Simpson, E. & Gordon, R. D. *Immun. Rev.* **35**, 59–75 (1977).
7. Zinkernagel, R. M., Dunlop, M. B. C., Blanden, R. V., Doherty, P. C. & Shreffler, D. C. *J. exp. Med.* **144**, 519–532 (1976).
8. Bubbers, J. E., Blank, K. J., Freedman, H. A. & Lilly, F. *Scand. J. Immun.* **6**, 533–539 (1977).
9. Trinchieri, G., Bernoco, D., Curtoni, S. E., Miggianno, V. C. & Ceppellini, R. in *Histocompatibility Testing 1972*, 510–519 (Munksgaard, Copenhagen, 1973).
10. Simonsen, M. *Transplant. Rev.* **3**, 22–35 (1970).

## Conventional calcium channel mediates asynchronous acetylcholine release by motor nerve impulses

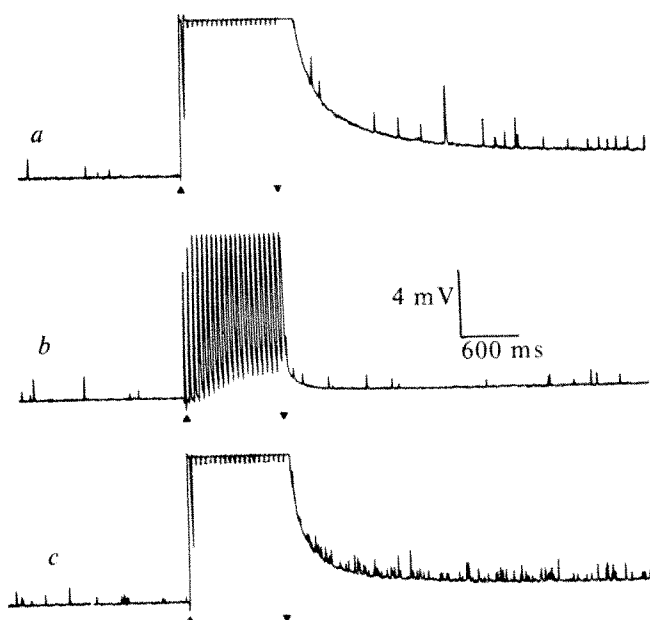
STIMULATION of the motor nerve, in addition to producing the synchronous, impulsive release of acetylcholine (ACh) that is recorded electrophysiologically as the endplate potential (e.p.p.)<sup>1</sup>, also elicits a delayed, asynchronous discharge of ACh quanta that appears as increases in miniature endplate potential (m.e.p.p.) frequencies<sup>2–4</sup>. The synchronous release of ACh is mediated by the movement of  $\text{Ca}^{2+}$  through specific conductance channels activated by depolarisation of the motor nerve ending (for reviews see refs 1, 5 and 6). Although asynchronous release is dependent in some fashion on extracellular ( $\text{Ca}^{2+}$ ) (ref. 4) there seems to be a controversy as to the precise nature of the ionic pathway responsible for this dependence. For example, although it has been suggested that asynchronous evoked release is mediated by residual  $\text{Ca}^{2+}$  that enters through the traditional  $\text{Ca}^{2+}$  conductance pathway<sup>3</sup>, results with  $\text{Mg}^{2+}$  have suggested the contrary<sup>4</sup>. Specifically, it has been shown that  $\text{Mg}^{2+}$ , rather than antagonising the asynchronous release of ACh in  $\text{Ca}^{2+}$  solutions, (as would be expected if  $\text{Ca}^{2+}$  moved through the same conductance pathway for both forms of release<sup>7–10</sup>), actually enhanced the asynchronous discharge of ACh quanta<sup>4</sup>. The present study investi-

gated the effects of the conventional  $\text{Ca}^{2+}$  antagonists,  $\text{Co}^{2+}$  and  $\text{Mg}^{2+}$ , on the asynchronous evoked release of ACh. The results demonstrate that after brief, repetitive stimulation, both ions competitively antagonise asynchronous ACh release in a manner similar to their respective antagonism of synchronous release<sup>7,10</sup>. We conclude that  $\text{Ca}^{2+}$  supports both dispersed and synchronous ACh release through the same conductance pathway.

Experiments were carried out using isolated nerve-cutaneous pectoris preparations of the frog, which were bathed in normal Ringer solution of the following composition (mM): NaCl 115, KCl 2,  $\text{NaHCO}_3$  2 and  $\text{CaCl}_2$  1.8 (pH 6.8–7.2). Neostigmine methylsulphate ( $1 \mu\text{g ml}^{-1}$ ) was added to all solutions to increase the size of the m.e.p.p.s. In most experiments, after an initial wash in normal Ringer, 400 mM glycerol was added to the normal Ringer (glycerol Ringer) and the preparation bathed in glycerol Ringer for 1 h. When the preparation is returned to normal Ringer after glycerol treatment, the transverse tubule system of the muscle ruptures<sup>11</sup>. This procedure allowed evoked ACh release to be studied in a full range of  $\text{Ca}^{2+}$  concentrations without fear of dislodging the recording electrode mechanically from the interior of the muscle fibre. Intracellular recordings were obtained from endplate regions using microelectrodes filled with 3 M KCl (resistances ranging from 10–25 MΩ). The signal from the microelectrode was fed into a conventional preamplifier (W. P. Instruments) and then in parallel into an oscilloscope (Tektronix model 5103N) and a pen recorder (Brush-Gould model 220). M.e.p.p. frequencies were measured from pen records. In the presence of  $\text{Ca}^{2+}$ , stimuli were delivered to the motor nerve at a frequency of 20 Hz (for 0.5–5 s) and the evoked



**Fig. 1** Competitive antagonism of  $\text{Ca}^{2+}$ -mediated asynchronous release by  $\text{Co}^{2+}$ . Stimuli were delivered at 20 Hz for 800 ms ( $\blacktriangle$ ). The preparation was pretreated with glycerol Ringer. Evoked m.e.p.p. frequencies were determined during the 4-s period immediately after nerve stimulation. All figures are photographs of pen recorder traces. *a*, Normal (1.8 mM  $\text{Ca}^{2+}$ ) Ringer. During nerve stimulation large e.p.p.s are produced (which are superimposed on the underlying depolarisation produced by residual ACh). After nerve stimulation, a residual discharge of individual m.e.p.p.s occurs. For *a*, *b* and *c*, resting m.e.p.p. frequency was  $2 \text{ s}^{-1}$ . Constancy of resting m.e.p.p. frequency was maintained by adding appropriate amounts of sucrose to the Ringer solution. In *a* the m.e.p.p. frequency after stimulation was  $4.4 \text{ s}^{-1}$ ; *b*, 1.8 mM  $\text{Ca}^{2+} + 0.5 \text{ mM Co}^{2+}$  Ringer, note the antagonism of both e.p.p. amplitude and evoked m.e.p.p. discharge by  $\text{Co}^{2+}$ . M.e.p.p. frequency after stimulation  $2 \text{ s}^{-1}$ ; *c*, 7.0 mM  $\text{Ca}^{2+} + 0.5 \text{ mM Co}^{2+}$  note partial surmounting of  $\text{Co}^{2+}$ -induced depression by increasing the  $[\text{Ca}^{2+}]$ . M.e.p.p. frequency after stimulation  $3.1 \text{ s}^{-1}$ . In this experiment, 9.0 mM  $\text{Ca}^{2+} + 0.5 \text{ mM Co}^{2+}$  Ringer produced a matching evoked m.e.p.p. discharge to that produced in normal Ringer.



**Fig. 2** Antagonism of  $\text{Ca}^{2+}$ -mediated, asynchronous release by  $\text{Mg}^{2+}$ . The preparation was pretreated with glycerol Ringer. Stimulation ( $\blacktriangle$ ) was delivered at a frequency of 20 Hz for 1 s. *a*, 1.8 mM  $\text{Ca}^{2+}$  Ringer resting m.e.p.p. frequency,  $2.4 \text{ s}^{-1}$ , evoked m.e.p.p. frequency,  $5.9 \text{ s}^{-1}$ ; *b*, 1.8 mM  $\text{Ca}^{2+}$  + 10 mM  $\text{Mg}^{2+}$  Ringer, resting m.e.p.p. frequency,  $3.9 \text{ s}^{-1}$ , evoked m.e.p.p. frequency,  $4.1 \text{ s}^{-1}$ , note the antagonism of evoked m.e.p.p.s by  $\text{Mg}^{2+}$ ; *c*, 6.25 mM  $\text{Ca}^{2+}$  + 10 mM  $\text{Mg}^{2+}$  Ringer, resting m.e.p.p. frequency,  $5.8 \text{ s}^{-1}$  evoked m.e.p.p., frequency  $11.6 \text{ s}^{-1}$ . Antagonism was surmounted by increasing the  $[\text{Ca}^{2+}]$ . For further explanation, see Fig. 1.

m.e.p.p. frequencies measured for the 4-s period after stimulation. During stimulation, a large depolarisation of the postsynaptic membrane (produced by inadequate removal of the enormous quantities of ACh poured into the synaptic cleft<sup>12</sup>) drove the recorder pen off the scale (see, for example, Figs 1 and 2). To ensure faithful counting of m.e.p.p. frequencies during repolarisation after stimulation, either (1), one output of the recorder was differentially biased with respect to the other, so that when the pen had moved off the scale on the lower part of one channel, it appeared on the scale at the upper part of the other channel or (2), the output terminal of the a.c.-coupled oscilloscope amplifier was fed directly into the recorder.

Figure 1 illustrates a typical experimental result from a preparation pretreated with glycerol Ringer. In Fig. 1*a*, repetitive nerve stimulation in normal Ringer (1.8 mM  $\text{Ca}^{2+}$ ) produced e.p.p.s superimposed on a depolarising membrane potential and a residual discharge of m.e.p.p.s after the stimulation period. In this trace, the m.e.p.p. frequency increased from a resting level of  $2 \text{ s}^{-1}$  to approximately  $4.5 \text{ s}^{-1}$  after stimulation. The addition of 0.5 mM  $\text{Co}^{2+}$  to normal Ringer (Fig. 1*b*) depressed the e.p.p. amplitude and fully antagonised the evoked discharge of m.e.p.p.s. Increasing the extracellular  $\text{Ca}^{2+}$  to 7.0 mM in the presence of  $\text{Co}^{2+}$  (Fig. 1*c*) enabled the antagonism of both the e.p.p. amplitude and evoked m.e.p.p. frequency to be overcome. In this experiment, a concentration of 9.0 mM  $\text{Ca}^{2+}$  was necessary to produce an evoked discharge of m.e.p.p.s that matched the control response (Fig. 1*a*). Note that in this experiment, changes in the resting m.e.p.p. frequencies (as a consequence of tonicity changes of the various Ringer solutions) were prevented by the addition of appropriate amounts of sucrose to the Ringer. In several experiments it was possible to construct a log dose-response curve for  $\text{Ca}^{2+}$ -evoked m.e.p.p.s and to observe a parallel shift in this curve in the presence of  $\text{Co}^{2+}$ . This suggests that  $\text{Co}^{2+}$  acts as a competitive antagonist of asynchronous

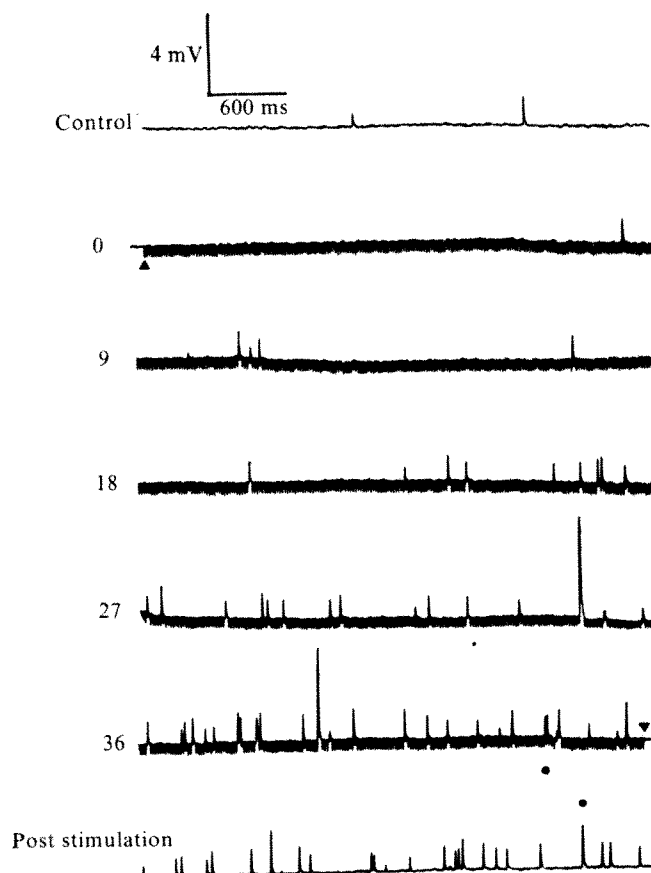
release<sup>8,12</sup>, much as it does for synchronous release<sup>10,13</sup>. The equilibrium dissociation constant ( $K_d$ ) for  $\text{Co}^{2+}$  as a competitive antagonist of asynchronous release can be calculated as follows<sup>8,13</sup>

$$K_d = ((\text{Ca}_2/\text{Ca}_1) - 1)^{-1} [\text{antagonist}] \quad (1)$$

where  $\text{Ca}_1$  is the  $[\text{Ca}^{2+}]$  that produces a certain evoked discharge of m.e.p.p.s and  $\text{Ca}_2$  is the  $[\text{Ca}^{2+}]$  that produces an identical evoked discharge of m.e.p.p.s in the presence of the antagonist. In four experiments in which the resting m.e.p.p. frequency remained essentially unchanged, the  $K_d$  for  $\text{Co}^{2+}$  ranged from 0.10 to 0.15 mM with a mean for all experiments of 0.13 mM. This value compares favourably with the reported values of  $\text{Co}^{2+}$  as an antagonist of synchronous release (0.07 mM (ref. 10), 0.18 mM (ref. 14)) suggesting that both forms of release may share a common conductance pathway.

Further evidence in support of this contention was provided by experiments using  $\text{Mg}^{2+}$ . In the experiment shown in Fig. 2, stimulation in normal Ringer after glycerol treatment produced a large increase in m.e.p.p. frequency from a resting level of  $2.4 \text{ s}^{-1}$  to  $6 \text{ s}^{-1}$  after stimulation. Addition of 10 mM  $\text{Mg}^{2+}$ , although causing a slight increase in resting frequency to  $3.9 \text{ s}^{-1}$  (presumably because of increased tonicity of the Ringer), fully antagonised the evoked discharge of m.e.p.p.s. Increasing the  $[\text{Ca}^{2+}]$  to 6.25 mM,

**Fig. 3** Slow increases in m.e.p.p. frequency evoked by prolonged (40 s) stimulation at 50 Hz in 10 mM  $\text{Mg}^{2+}$  Ringer. Upper trace, control m.e.p.p. frequency,  $0.5 \text{ s}^{-1}$ ; Middle traces, m.e.p.p. frequencies at indicated times after starting stimulation. Spikes on baseline indicates stimulus artefacts. Lowest trace, m.e.p.p. frequency immediately after stimulation,  $6 \text{ s}^{-1}$ . Note that a discernable increase in m.e.p.p. frequency begins only after 18 s of stimulation. Similar records were produced in the presence of 1 mM  $\text{Co}^{2+}$ .



although further increasing the resting frequency, overcame the antagonism of evoked m.e.p.s by  $Mg^{2+}$ . Experiments where the change in resting m.e.p.p. frequency was prevented by adding sucrose to the Ringer solutions were used to calculate the  $K_d$  for  $Mg^{2+}$  (equation 1). Indeed, the mean  $K_d$  for  $Mg^{2+}$  is  $4.6 \pm 0.2$  mM (mean  $\pm$  s.e.,  $n=4$ ) as an antagonist of asynchronous release is similar to the values for  $Mg^{2+}$  as an antagonist of synchronous release (4.0 mM, 3.0 mM, 4.4 mM: refs 8–10 respectively).

These results, demonstrating that  $Mg^{2+}$  antagonises the asynchronous  $Ca^{2+}$ -dependent discharge of m.e.p.s seem to be at variance with those suggesting that  $Mg^{2+}$  enhances the asynchronous release of ACh. It is possible that the ability of  $Mg^{2+}$  to activate asynchronous evoked release may appear only after prolonged, high frequency stimulation. Figure 3 illustrates a representative experiment which suggests that this is the case. Note that it is only after 18 s of high frequency (50 Hz) stimulation in 10 mM  $Mg^{2+}$  Ringer that discernable increases in m.e.p.p. frequency were observed. This is in contrast to the results shown in Figs 1 and 2, where stimuli were delivered at a frequency of 20 Hz for a brief period ( $\leq 1$  s). In the experiment of Fig. 3 m.e.p.p. frequencies were elevated from the control level of  $0.5 s^{-1}$  (upper trace) to  $6 s^{-1}$  immediately after 40 s of stimulation (lowest trace). A concentration of  $Co^{2+}$  as high as 1 mM did not alter either the time course or the absolute increase in m.e.p.p. frequency produced by stimulation in 10 mM  $Mg^{2+}$  Ringer. It thus seems that  $Ca^{2+}$  and  $Mg^{2+}$  support asynchronous release through different ionic conductance channels<sup>4,15</sup>.

In conclusion, the results suggest that the  $Ca^{2+}$ -dependent asynchronous release of ACh by nerve stimulation is mediated by the same conductance pathway as synchronous release. It is interesting that  $Ba^{2+}$  (which does not support synchronous release<sup>16</sup>) and  $Sr^{2+}$  (which can only poorly support synchronous release<sup>17</sup>) both produce large discharges of m.e.p.s after brief repetitive nerve stimulation. In contrast to  $Mg^{2+}$ , however, both  $Ba^{2+}$  and  $Sr^{2+}$  support asynchronous release through the same conductance pathway normally traversed by  $Ca^{2+}$  in mediating the synchronous and asynchronous discharge of ACh quanta (ref. 18 and our work in preparation).

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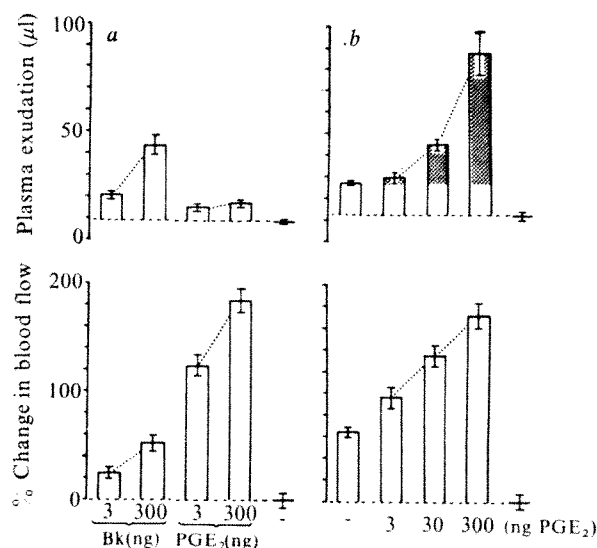
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- Katz, B. *Release of Neural Transmitter Substances* (University Press, Liverpool, 1969).
- del Castillo, J. & Katz, B. *J. Physiol., Lond.* **124**, 574–585 (1954).
- Miledi, R. & Thies, R. *J. Physiol., Lond.* **212**, 245–257 (1971).
- Hurlbut, W. P., Longnecker, H. B. & Mauro, A. *J. Physiol., Lond.* **219**, 17–38 (1971).
- Hubbard, J. I. *Physiol. Rev.* **53**, 674–723 (1973).
- Ginsborg, B. L. & Jenkinson, D. H. in *Neuromuscular Junction, Handbook of Experimental Pharmacology* (ed. Zaimis, E.) 229–364 (Springer Verlag, New York, 1976).
- del Castillo, J. & Katz, B. *J. Physiol., Lond.* **124**, 560–573 (1954).
- Jenkinson, D. H. *J. Physiol., Lond.* **138**, 434–445 (1957).
- Dodge, F. A., Jr & Rahamimoff, R. *J. Physiol., Lond.* **193**, 419–432 (1967).
- Crawford, A. C., *J. Physiol., Lond.* **240**, 255–278 (1974).
- Eisenberg, R. S., Howell, J. N. & Vaughan, P. C. *J. Physiol., Lond.* **215**, 95–102 (1971).
- Katz, B. & Miledi, R. *Proc. R. Soc. Lond.* **B192**, 27–38 (1975).
- Gaddum, J. H. *Pharmac. rev.* **9**, 211–218 (1957).
- Weakly, J. N. *J. Physiol., Lond.* **234**, 597–612 (1973).
- Baker, P. F. *Progr. Biophys. molec. Biol.* **24**, 177–223 (1972).
- Silinsky, E. M. *Br. J. Pharmac.* **59**, 215–217 (1977).
- Dodge, F. A., Jr., Miledi, R. & Rahamimoff, R. *J. Physiol., Lond.* **200**, 267–283 (1969).
- Silinsky, E. M. *J. Physiol., Lond.* (in the press).

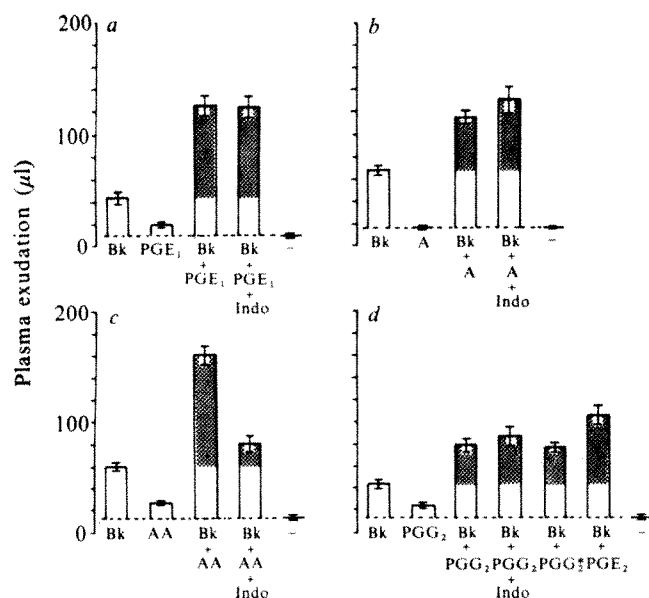
## Role of prostaglandin-mediated vasodilatation in inflammation

INFLAMMATORY reactions are characterised by hyperaemia, and exudation of plasma resulting in tissue swelling. Putative mediators of inflammation have usually been evaluated according to their ability to mimic inflammatory reactions<sup>1</sup>. Although prostaglandins were identified in inflammatory exudates<sup>2</sup>, and prostaglandin synthesis *in vitro* was shown to be inhibited by non-steroid anti-inflammatory compounds<sup>3–5</sup>, prostaglandins (unlike histamine and bradykinin) were found to be poor at eliciting plasma exudation when injected into guinea pig<sup>6–7</sup> or rabbit skin<sup>8</sup>. But, the finding that exogenous prostaglandins (notably of the E-type) potentiate plasma exudation produced by other mediators<sup>7–11</sup> suggested that this may be the role of prostaglandins in inflammation. An alternative view has been proposed by Kuehl *et al.*<sup>12</sup>, who consider that since E and F prostaglandins fail fully to mimic inflammatory reactions, other products of arachidonic acid should be considered as mediators. They suggest that the unstable prostaglandin endoperoxide,  $PGG_2$ , or a non-prostaglandin product of it (a free radical could be the important mediator. We have re-examined the mode of action of prostaglandins in inflammation by measuring both increased blood flow and plasma exudation. The results presented here



**Fig. 1.** Plasma exudation (upper) and local blood flow changes (lower) measured simultaneously in rabbit skin in response to various agents. In each experiment a New Zealand White rabbit was given an intravenous injection (approximately 15  $\mu$ Ci per kg body weight) of  $^{131}I$ -labelled human serum albumin and a short-acting anaesthetic, methohexitone sodium (approximately 10 mg per kg). Intradermal injections (0.1 ml) of inflammatory agents mixed with  $^{133}Xe$  (5–10  $\mu$ Ci per injection) were then given into the clipped back skin in random-block order. After 20 min the animal was killed, skinned, and a 16-mm diameter punch was used to remove injection areas. Samples of skin, blood, and injection fluid were counted in an automatic  $\gamma$ -counter. Plasma volumes of skin samples ( $\mu$ l per sample) and local blood changes (% increase above controls) were computed as previously described<sup>16</sup>. *a*, Comparison of the responses produced by bradykinin (Bk) and  $PGE_2$  at the doses shown. Bradykinin produced exudation with little blood flow increase. Conversely,  $PGE_2$  produced little exudation but a large increase in blood flow. *b*, Effect of adding increasing doses of  $PGE_2$  to a fixed dose of histamine (H, 2.5  $\mu$ g) resulting in an increase in blood flow and a potentiation of exudation. In both *a* and *b* the shaded areas denote the amount of exudation produced above that produced by the permeability-increasing agent alone; dashed lines represent saline control level for both plasma volume and blood flow; single dash represents saline injection. Values are means  $\pm$  s.e.m.,  $n = 6$ .



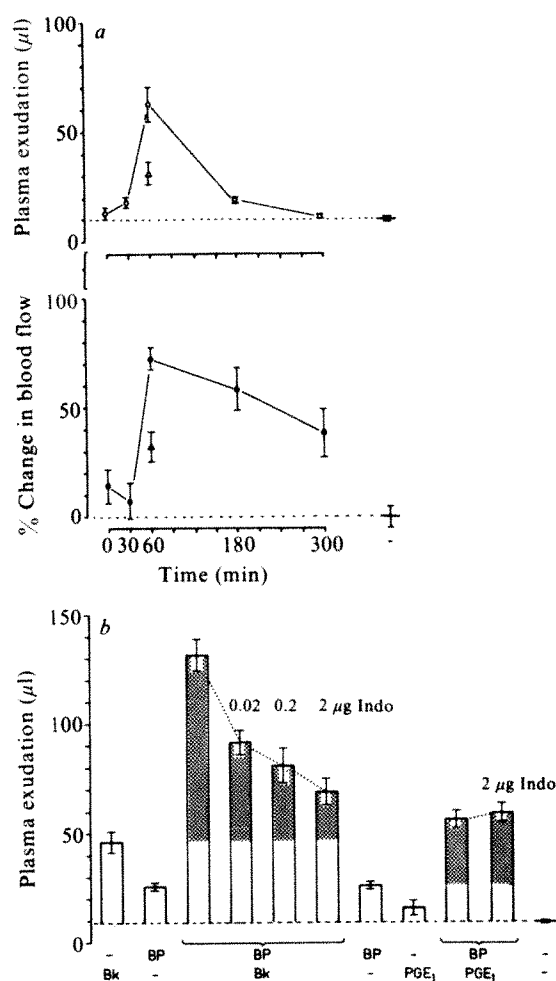


**Fig. 2** Four experiments showing potentiation of exudation responses to bradykinin (Bk, 500 ng) by: PGE<sub>1</sub> (10 ng); adenosine (A, 100  $\mu\text{g}$ ); arachidonate (AA, 1  $\mu\text{g}$ ); and PGG<sub>2</sub> (100 ng). Addition of 1  $\mu\text{g}$  indomethacin (Indo) suppressed only the potentiation produced by arachidonate. Incubation of PGG<sub>2</sub> in saline at 37 °C for 10 min (PGG<sub>2</sub>\*) did not reduce its potentiating activity. Potentiation produced by the same dose (100 ng) of PGE<sub>2</sub> is shown for comparison. Values are means  $\pm$  s.e.m.,  $n = 6$ .

suggest that the mediation of vasodilatation and increased vascular permeability should be considered separately. Prostaglandins (notably the E-type<sup>13,14</sup>) mediate vasodilatation (although they may have some additional mast cell degranulating activity in the rat<sup>15</sup>). It is this vasodilatation which is responsible for the potentiation of the exudation produced by other mediators. Our observations provide a new hypothesis for the microvascular mechanisms involved in the action of non-steroid anti-inflammatory compounds.

Plasma exudation and local blood flow changes were measured in rabbit skin using the accumulation of intravenously-injected <sup>131</sup>I-albumin and wash-out of intradermally-injected <sup>133</sup>Xe, as previously described<sup>16</sup>.

Figure 1a shows that intradermal injection of E-type prostaglandin produced a large increase in local blood flow with little plasma exudation (often undetectable). Bradykinin (and histamine at larger doses), on the other hand, increased vascular permeability resulting in plasma exudation (for 10–15 min following injection<sup>17</sup>), but they were far less potent at increasing blood flow. An example of the exudation-potentiating activity of prostaglandins is shown in Fig. 1b, which demonstrates that addition of increasing doses of PGE<sub>2</sub> to a fixed dose of histamine resulted in increasing blood flow and an enhancement of exudation. We have previously reported<sup>8</sup> that exudation potentiation correlates with the vasodilator activity of prostaglandins, and a similar relationship has been observed using other techniques<sup>18</sup>. First, the rank order of different prostaglandins was the same for exudation-potentiating potency as for blood flow-increasing potency<sup>8</sup>, that is, PGE > PGA > PGF > PGD. Second, local injection of substances, other than prostaglandins, which increased skin blood flow, for example isoprenaline, ADP, and adenosine (see Fig. 2b), also had exudation-potentiating activity<sup>8</sup>. Conversely, substances which reduced skin blood flow, for example noradrenaline and angiotensin II, reduced exudation and blood flow in parallel<sup>8</sup>. From these results we conclude that prostaglandins increase histamine- and bradykinin-induced plasma exudation not by increasing vascular permeability, but by increasing vasodilatation. The exact mechanism of this is not clear, but extrapolation from observations on the transport of



**Fig. 3** a, Time course of exudation (○), and blood flow changes (●) produced by intradermal injection of killed *B. pertussis* vaccine ( $2.5 \times 10^8$  organisms per site) into an unsensitised rabbit. Responses varied between animals; for example exudation, mean  $23.1 \pm 3.0 \mu\text{l}$ , range 3.6–71.9  $\mu\text{l}$ ,  $n = 32$  rabbits (six measurements per animal). Abscissa is time between *B. pertussis* injections and following injections of isotopes. Exudation and blood flow were measured over a further 20 min period. Indomethacin (1  $\mu\text{g}$ ), mixed with the vaccine before injection reduced both blood flow increase (▲) and, therefore (according to the hypothesis), exudation (Δ). b, Enhanced exudation, at the peak of the response to intradermal *B. pertussis* injection, produced by a further injection of either bradykinin or PGE<sub>1</sub>. Potentiation of exudation produced by a first injection (scale top line) of *B. pertussis* (BP) and a second injection (lower line), 60 min later, of bradykinin (500 ng). Exudation was measured over the following 30 min. This effect was suppressed by locally-injected indomethacin (dose equally divided between first and second injections). The potentiation produced by a first injection of *B. pertussis* and a second injection of PGE<sub>1</sub> (500 ng) was unaffected by locally-injected indomethacin. Values are means  $\pm$  s.e.m.,  $n = 6$ .

macromolecules from blood to lymph in dog paw following thermal injury<sup>19</sup>, suggest that exudation potentiation results from an increase in total vessel wall exchange area in the dilated vascular bed.

Figure 2 shows potentiation of bradykinin-induced exudation by PGE<sub>1</sub>, adenosine, arachidonate<sup>20</sup>, and PGG<sub>2</sub>. Adenosine is included as an example of a non-prostaglandin vasodilator. All four agents produced little exudation when injected alone. Potentiation produced by PGE<sub>1</sub> (and PGE<sub>2</sub>, not shown), adenosine, and PGG<sub>2</sub>, was unaffected by locally-injected indomethacin; whereas that produced by arachidonate, the PGE<sub>2</sub> precursor (and similarly dihomo- $\gamma$ -linolenate, the PGE<sub>1</sub> precursor) was suppressed. These results are consistent with the proposition that indomethacin suppresses inflammatory dilatation, and as a consequence inflammatory oedema, by preventing the production of a vasodilator substance via the cyclo-oxygenase pathway. Although the importance of the unstable prostaglandin endoperoxide, PGG<sub>2</sub>, has been emphasised<sup>12</sup>, it is interesting to

note that  $\text{PGG}_2$  injected alone produced little exudation. Furthermore, the exudation-potentiating potency of  $\text{PGG}_2$  was less than that of  $\text{PGE}_2$  and was unaffected by incubation in saline for 10 min at  $37^\circ\text{C}$  (which would leave little  $\text{PGG}_2$  unchanged). This would suggest that the observed activity of  $\text{PGG}_2$  *in vivo* was due to one of its products,  $\text{PGE}_2$ . Indeed, although injection of  $\text{PGG}_2$  produced a net increase in blood flow over the period measured (20 min), other evidence based on the time course of  $^{133}\text{Xe}$  clearance<sup>21</sup> would suggest that the endoperoxides themselves may have vasoconstrictor activity.

The results obtained using exogenous agents suggest that inflammation may involve the separate production of permeability-increasing mediators and vasodilator mediators. This is supported by results from experiments using intradermal injections of killed *Bordetella pertussis* vaccine in unsensitized rabbits. Other materials produced similar results, for example Zymosan, glycogen and carrageenan. Intradermal injection of *B. pertussis* resulted in exudation and increased blood flow with a peak at 60–80 min (Fig. 3a). Indomethacin suppressed both exudation and increased blood flow (Fig. 3a). The presence of an endogenous vasodilator (exudation-potentiating) mediator was established by injecting bradykinin at the peak of the response, 60 min after *B. pertussis* injection. The exudation response to bradykinin was potentiated (Fig. 3b) and this effect was inhibited by locally-injected indomethacin. The presence of an endogenous permeability-increasing mediator (unlikely to be either histamine or a kinin; T.J.W., unpublished observations) was established by injection of  $\text{PGE}_1$  60 min after *B. pertussis* injection.  $\text{PGE}_1$  also produced potentiation (Fig. 3b) but this effect was unaffected by indomethacin. Thus, indomethacin inhibited the production of the vasodilator (exudation-potentiating) mediator(s), and not the production of the permeability-increasing mediator(s).

We conclude that vascular changes in inflammatory reactions should be considered in terms of two types of chemical mediators—mediators which are predominantly vasodilators and mediators which are important for their vascular permeability-increasing activity. The amount of plasma exudation is dependent on the level of both types of mediators; and it follows that oedema can be suppressed by inhibiting the production, or action, of either type. Permeability-increasing mediators seem to be independent of the cyclo-oxygenase pathway of arachidonate (or dihomo- $\gamma$ -linolenate) metabolism. No evidence was obtained in support of the recently suggested<sup>12</sup> pivotal role for  $\text{PGG}_2$ ; in inflammation the important substances derived from arachidonate (or dihomo- $\gamma$ -linolenate) are vasodilators, probably E-type prostaglandins (although the recently discovered  $\text{PGI}_2$  cannot be excluded from a similar role). Indomethacin was found to inhibit specifically the production of vasodilator mediators. Thus, we propose that non-steroid anti-inflammatory compounds reduce inflammatory oedema as a consequence of a suppression of vasodilatation.

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- Lewis, T. *The Blood Vessels of the Human Skin and their Responses* (Shaw, London, 1927).
- Willis, A. L. *J. Pharm. Pharmacol.* **21**, 126–128 (1969).
- Vane, J. R. *Nature new Biol.* **231**, 232–235 (1971).
- Smith, J. B. & Willis, A. L. *Nature new Biol.* **231**, 235–237 (1971).
- Ferreira, S. H., Moncada, S. & Vane, J. R. *Nature new Biol.* **231**, 237–239 (1971).
- Horton, E. W. *Nature* **200**, 892–893 (1963).
- Williams, T. J. & Morley, J. *Nature* **246**, 215–217 (1973).
- Williams, T. J. *Br. J. Pharmacol.* **56**, 341P–342P (1976).
- Moncada, S., Ferreira, S. H. & Vane, J. R. *Nature* **246**, 217–219 (1973).
- Thomas, G. & West, G. B. *J. Pharm. Pharmacol.* **25**, 747–748 (1973).
- Lewis, A. J., Nelson, D. J. & Sugrue, M. F. *Br. J. Pharmacol.* **55**, 51–56 (1975).
- Kuehl, F. A. *et al. Nature* **265**, 170–173 (1977).

- Berström, S., Duner, H., von Euler, U. S., Pernow, B. & Sjövall, J. *Acta physiol. scand.* **45**, 145–151 (1959).
- Solomon, L. M., Juhlin, L. & Kirschbaum, M. B. *J. invest. Dermatol.* **51**, 280–282 (1968).
- Crunkhorn, P. & Willis, A. L. *Br. J. Pharmacol.* **41**, 49–56 (1971).
- Williams, T. J. *J. Physiol., Lond.* **254**, 4P–5P (1976).
- Williams, T. J. & Morley, J. *Br. J. exp. Pathol.* **55**, 1–12 (1974).
- Johnston, M. G., Hay, J. B. & Movat, H. Z. *Agents Actions* **6**, 705–711 (1976).
- Arturson, G. & Jonsson, C.-E. *Uppsala J. Med. Sci.* **78**, 181–188 (1973).
- Ikeida, K., Tanaka, K. & Katori, M. *Prostaglandins* **10**, 747–758 (1975).
- Lewis, G. P., Westwick, J. & Williams, T. J. *Br. J. Pharmacol.* **59**, 442P (1977).

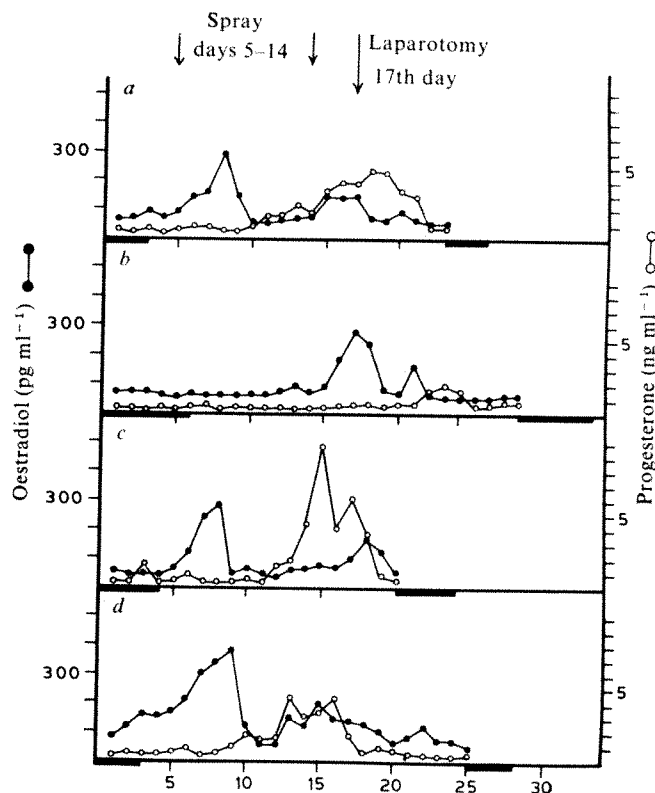
## Ovulation in rhesus monkeys suppressed by intranasal administration of progesterone and norethisterone

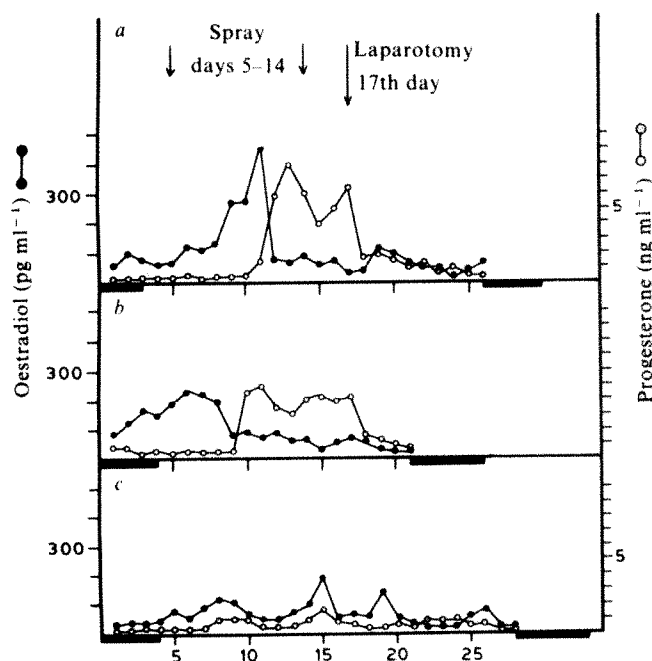
THE importance of various brain structures in the regulation of secretion of gonadotropins<sup>1–3</sup> and the presence of steroid hormones in the cerebrospinal fluid (CSF)<sup>4–5</sup> suggest that a method for delivering steroids into the CSF might be useful in the control of fertility. Steroids can enter the cerebral components rapidly after intranasal administration and their concentrations are higher than after intravenous injection<sup>6</sup>. We report here that progesterone and norethisterone given intranasally can prevent ovulation in rhesus monkeys.

Our studies were carried out between September and March during when almost all the monkeys in our colonies have ovulatory menstrual cycles. We used adult female rhesus monkeys (5–6 kg) which had experienced at least two successive ovulatory menstrual cycles of normal duration (20–33 d) (determined by monitoring circulating levels of progesterone throughout the menstrual cycles).

Two steroids, progesterone, a naturally occurring ovarian hormone and norethisterone, a synthetic progestogen widely used in hormonal contraception were

**Fig. 1** Effects of spraying progesterone intranasally in rhesus monkeys. Serum profiles of oestradiol and progesterone throughout the menstrual cycle are shown for one monkey representative of each of the four treated groups. Ovulation was suppressed only in the monkey given  $2\mu\text{g d}^{-1}$ . Black bars on x axis indicate menstruation. a, Control monkey no. 388; b, monkey no. 695,  $2\mu\text{g d}^{-1}$ ; c, monkey no. 690,  $10\mu\text{g d}^{-1}$ ; d, monkey no. 699,  $30\mu\text{g d}^{-1}$ .





**Fig. 2** Effects of spraying norethisterone intranasally in rhesus monkeys. Serum profiles of oestradiol and progesterone throughout the menstrual cycle are shown for one monkey representative of each of the three treated groups. Ovulation was suppressed only in the monkey given  $9 \mu\text{g d}^{-1}$ . Black bars on x axis indicate menstruation. *a*, Control monkey no. 705; *b*, monkey no. 703,  $3 \mu\text{g d}^{-1}$ ; *c*, monkey no. 695,  $9 \mu\text{g d}^{-1}$ .

dissolved in a mixture of ethanol:propylene glycol:water (1:1:3). A constant volume (0.5 ml) of this mixture was put into a glass atomiser (Brovon Inhaler, Moore Medicinal Products Ltd), which was inserted into one nostril of a conscious monkey seated on a restraining chair. The mixture was sprayed into the nostril for 10 s by connecting the atomiser to a nitrogen source regulated to deliver the inert gas at a constant pressure of  $0.25 \text{ kg cm}^{-2}$ . Before use, each spraying device was calibrated to deliver the drug for 10 s. The amount of steroid administered to each animal was regulated either by varying the concentration of the steroid in the solvent or by repeated spraying for periods of 10 s. Controls were sprayed with the solvent alone.

The effects on ovulation were determined by measuring serum levels of oestradiol and progesterone in blood samples taken daily throughout the menstrual cycle, as well as by visual observation of the ovaries after laparotomy on day 17 of the menstrual cycle. Circulating levels of progesterone below  $2 \text{ ng ml}^{-1}$  serum together with the regressed state of ovaries at laparotomy were taken as indicative that ovulation had been suppressed. It was assumed to have occurred when circulating levels of progesterone were greater than  $3 \text{ ng ml}^{-1}$  serum and up to  $5\text{--}6 \text{ ng ml}^{-1}$ , and when at the same time one of the ovaries was enlarged and had a newly formed corpus luteum.

Among monkeys treated with progesterone, ovulation was always suppressed by  $2 \mu\text{g d}^{-1}$  and also in two out of six cases when  $10 \mu\text{g d}^{-1}$  was given. Ovulation occurred in four of the six monkeys given  $10 \mu\text{g d}^{-1}$  as well as in all those given  $30 \mu\text{g d}^{-1}$  (Table 1, Fig. 1).

In the monkeys treated with norethisterone, ovulation was unaffected by  $3 \mu\text{g d}^{-1}$  but was suppressed in all cases when  $9$  or  $17 \mu\text{g d}^{-1}$  was given (Table 1, Fig. 2).

These results show that intranasal administration of steroids can suppress ovulation. The two steroids had different effects: progesterone suppressed ovulation at the lowest dose but not in all animals at higher doses. Norethisterone was effective only at the two higher doses. The

**Table 1** Effect of spraying steroid hormones intranasally during days 5–14 of menstrual cycle

Treatment	Menstrual cycle length (d)			
	Monkey no.	Ovulation	Untreated	Treated
Controls	388	+	31	23
	630	+	25	25
	687	+	30	30
	694	+	24	23
	705*	+	26	26
	717*	+	24	23
Progesterone	673	—	27	21
	688	—	22	29
	DD, $2 \mu\text{g}$	—	23	27
	703*	—	26	29
	704	—	25	33
	727*	—	28	31
Group B	655	—	29	23
	DD, $10 \mu\text{g}$	—	24	20
	695*	+	25	24
	696*	+	31	22
	700	—	30	50
	717	+	33	27
Group C	699	+	25	21
	DD, $30 \mu\text{g}$	+	26	30
	728	+	23	26
Norethisterone				
	Group D			
DD, $3 \mu\text{g}$	703*	+	24	21
	TD, $30 \mu\text{g}$			
Group E				
	DD, $9 \mu\text{g}$	—	26	28
	696*	—	24	28
	705*	—	26	28
Group E				
	DD, $17 \mu\text{g}$	—	21	113
	TD, $170 \mu\text{g}$	—	23	65

Controls were sprayed with solvent only. DD, daily dose; TD, total dose; +, occurrence of ovulation; —, no ovulation.

\*At least two successive ovulatory menstrual cycles of normal duration were observed in those animals before they were used in their experiments.

different effects of progesterone is not surprising in view of its ability to inhibit or facilitate gonadotropin secretion depending on circumstances. When administered to monkeys after oestradiol, it inhibits the oestrogen-induced surge of luteinising hormone (LH)<sup>7</sup>; if given in conjunction with oestradiol, it significantly advances this surge<sup>8</sup>. A facilitatory effect of progesterone on LH secretion has also been demonstrated in women<sup>9,10</sup>. In contrast, there is evidence that progesterone inhibits gonadotropin secretion and thus blocks ovulation in monkeys<sup>7,11</sup> and women<sup>12</sup>.

Further work is needed to explain the two dose-related, pharmacological effects of progesterone which we have observed.

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1. Anand Kumar, T. C. & Knowles, F. *Nature* **215**, 54–55 (1967).
2. Knowles, F. & Anand Kumar, T. C. *Phil. Trans. R. Soc., Lond.* **B256**, 357–375 (1969).



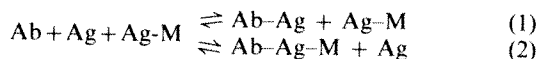
3. David, G. F. X., Umberkoman, B., Kumar, K. & Anand Kumar, T. C. in *Brain-Endocrine Interaction 2* (eds Knigge, K. M., Scott, D. E., Kobayashi, H. & Ishii, S.) 365-375 (Karger, Basel, 1975).
4. Anand Kumar, T. C. & Thomas, G. H. *Nature* **219**, 628-629 (1968).
5. David, G. F. X. & Anand Kumar, T. C. *Neuroendocrinology* **14**, 114-120 (1974).
6. Anand Kumar, T. C., David, G. F. X., Umberkoman, B. & Saini, K. D., *Curr. Sci.* **43**, 435-439 (1974).
7. Knobil, E. *Rec. Prog. Horm. Res.* **30**, 1-46 (1974).
8. Clifton, D. K., Steiner, R. A., Resko, J. A. & Spies, H. G. *Biol. Reprod.* **13**, 190-194 (1975).
9. Odell, W. D. & Swerdloff, R. S. *Proc. natn. Acad. Sci. U.S.A.* **61**, 529-536 (1968).
10. Nillius, S. J. & Wide, L. J. *Obstet. Br. Commonwealth* **78**, 822-827 (1971).
11. Spies, H. G. & Niswender, G. D. *Endocrinology* **90**, 257-261 (1972).
12. Netter, A., Gorius, A. & Joubinaux, J. *Annls Endocr.* **34**, 430-435 (1973).

## Metalloimmunoassay

THE disadvantages encountered with radioisotopes, such as high costs, health hazards, limited variety of useable isotopes, short shelf-life of the labelled antigens and difficulties of introducing the radioactive isotope on to the antigen molecule, have stimulated the search for non-isotopic methods in immunological work<sup>1</sup>. We report here on the feasibility of a novel non-isotopic system for immunochemical studies and its possible use in immunoassays. The basic concept of the new system is the use of metal atoms in the form of their organometallic or co-ordination complexes as labelling agents for haptens or macromolecular antigens.

The extensive chemistry of transition metal-organic complexes developed during the past two decades should provide a rich and versatile source for the synthesis of 'tailor-made' metal-labelled antigens, which would be free of the disadvantages of radioisotopes. The metal-labelled antigens are designated metallo-antigens and/or metallohapten and we suggest that the immunoassay based on these reagents be called metalloimmunoassay (MIA).

The basic requirements for a metalloimmunoassay are (1) preparation of reagents (antibodies and metallohapten) and (2) development of a method for quantifying the metal content. The metallohapten (Ag-M) and the unlabelled antigen (Ag) react with the specific antibodies, according to the competitive protein-binding equations (1) and (2).



After separation of the free, unbound antigen (Ag-M, Ag) from the antibody-antigen complexes (Ab-Ag-M, Ab-Ag), the amount of metal present in the bound (B) or free phase (F) can be determined by suitable analytical methods such as emission, absorption and fluorescence spectrometry, electrochemical methods and neutron activation. Preparation of a calibration curve plotted for standardised amounts of metallohapten and unlabelled antigen provides the means of determining the quantity of analysed substance in unknown samples.

Typical examples of reagent preparations performed during development of a metalloimmunoassay are illustrated in Fig. 1. The oestrogen steroids, oestrone (Ia), oestradiol-17 $\beta$  (Ib) and oestriol-16 $\alpha$ , 17 $\beta$  (Ic) were transformed in high yields (>80%) into their respective 3-O-carboxymethyl derivatives (IIa-c), as well as into oestradiol-17 $\beta$  hemisuccinate (IVb) and oestriol-16 $\alpha$ , 17 $\beta$ -hemisuccinate (IVc). These carboxylic acid derivatives were used for the preparation of bovine serum albumin (BSA) conjugates. The production of antisera (in rabbits) and determination of their titre and specificity by free radical immunoassay<sup>2</sup> were carried out as described previously<sup>3</sup>. The antibodies were insolubilised by reaction with cyanogen bromide-activated Sepharose 4B (Pharmacia)<sup>4</sup> in order to provide a convenient method for separation of free, unbound Ag-M from the antibody-antigen complex.

The same carboxylic acid derivatives (IIa-c and IVb-c) as well as the amino derivatives III and IVa were labelled with various metal complexes to form the oestrogen-metallohapten some of which are shown in Fig. 2. Also shown are examples of cannabinoid and barbiturate metallohapten. The structures of the compounds

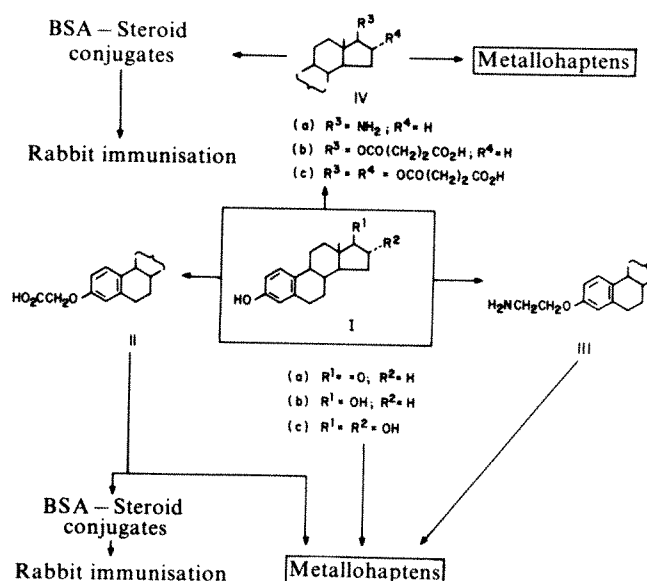
reported here were characterised by routine analytical procedures. BSA, as a representative protein molecule, has been labelled with both iron and manganese complexes, in a 1:20 molar ratio BSA:metal complex.

Atomic absorption spectrometry<sup>5</sup> was used for detection and quantification of metal concentrations in solutions containing metallohapten. The use of flameless-operation instruments (Perkin Elmer 403 with HGA-70 graphite furnace and Instrumentation 351 with 455 flameless unit and programable calculation data system kit 42168-02) enabled us to obtain satisfactory calibration curves in the metal concentration range 2-50 ng ml<sup>-1</sup>. This corresponds to unlabelled hapten concentrations in the range 20-500 ng ml<sup>-1</sup>, since the metal content is on the average about 10-20% of the molecular weight of the metallohapten. This concentration range is suitable for the detection of a relatively large number of urinary metabolites such as oestriol (in pregnancy urines), morphine, barbiturates, amphetamine and cocaine, without previous extraction and concentration operations. But, in this early stage of development and given the currently commercially available atomic absorption spectrometers, MIA cannot be used for the detection of picogram hapten concentrations, as can be achieved with radioimmunoassays<sup>6</sup>. Further work may improve detection limits.

Some indication of methodology can be obtained from a brief description of one of the procedures using reagents mentioned above. Standardised solutions of the iron-containing oestradiol-17 $\beta$ -succinate metallohapten, VII, in 0.01 M phosphate buffer (pH 7.3) with 15% dimethylformamide, yielded an atomic absorption calibration curve, in the 0-50 ng Fe ml<sup>-1</sup> concentration range, with a linear regression equation  $y = 1.731x + 42.88$  and a correlation coefficient  $r = 0.9977$ . A Sepharose-bound anti-oestradiol-17 $\beta$  hemisuccinate antibody preparation was titrated with standard solutions of metallohapten, VII, Vortex-mixed, incubated (30 min at room temperature) and centrifuged (1 min at 3,000 r.p.m.).

Aliquots (20-30  $\mu$ l) from the supernatant were injected into the graphite furnace of the atomic absorption spectrometer to measure the amount of free metallohapten, Ag-M. To determine inhibition (%) of metallohapten binding, a similar experiment was carried out using a constant concentration of metallohapten VII and increasing added amounts of unlabelled steroid IVb. With the

Fig. 1 Outline for synthesis of oestrogen haptens, their protein conjugates and metallohapten.



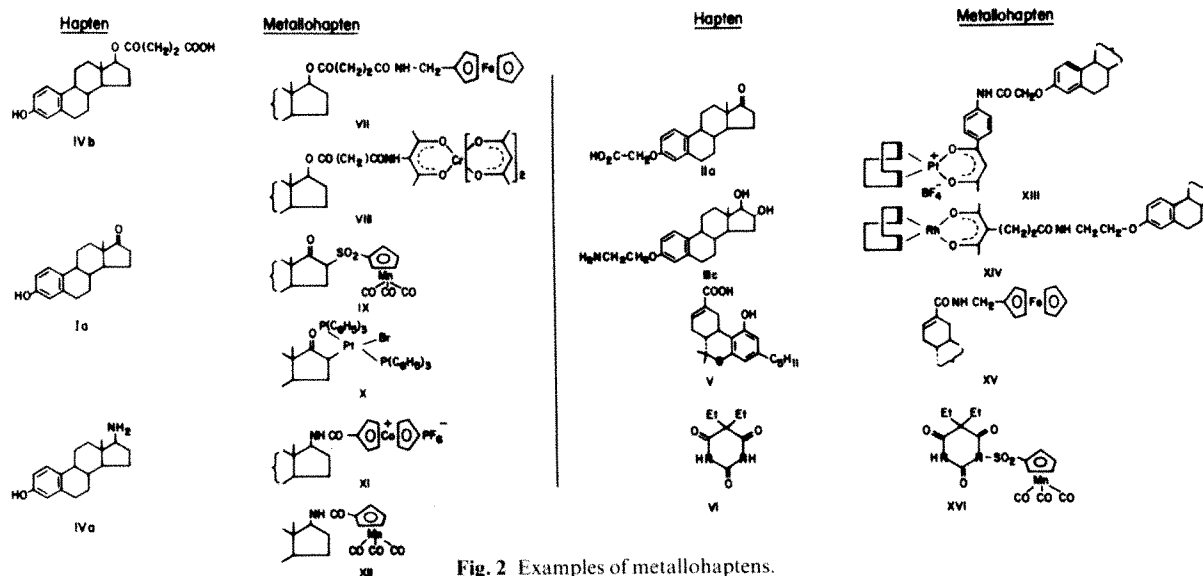


Fig. 2 Examples of metallohaptenes.

antisera used in this experiment and hapten concentrations of  $0.1\text{--}1\ \mu\text{g ml}^{-1}$ , a molar ratio unlabelled steroid to metallohapten of 1.31:1 was required to obtain about 44% inhibition of metallohapten binding. These and similar data demonstrate the feasibility of the concept and preliminary efforts to optimise the assay conditions have been promising.

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1. Landon, J., Crookall, J. & McGregor, A. in *Steroid Immunoassay* (eds Cameron, E. H. D., Hillier, S. G. & Griffith, K.) 183–188 (Alpha Omega, Cardiff, 1975).
2. Leute, R. K., Ullman, E. F., Goldstein, A. & Herzenberg, L. A. *Nature new Biol.* **236**, 93–95 (1972).
3. Cais, M., Dani, S., Josephy, Y., Modiano, A., Gershon, H. & Mechoulam, R. *FEBS Lett.* **55**, 257–260 (1975).
4. Cuatrecasas, F. & Anfinsen, C. B. *Meth. Enzym.* **22**, 345–378 (1971).
5. Price, W. J. *Analytical Atomic Absorption Spectrometry* (Heyden, London, New York and Rheine, 1974).
6. *Methods of Hormone Radioimmunoassay* (eds Jaffe, B. M. & Behrman, H. R.) 473–476 (Academic, New York and London, 1974).

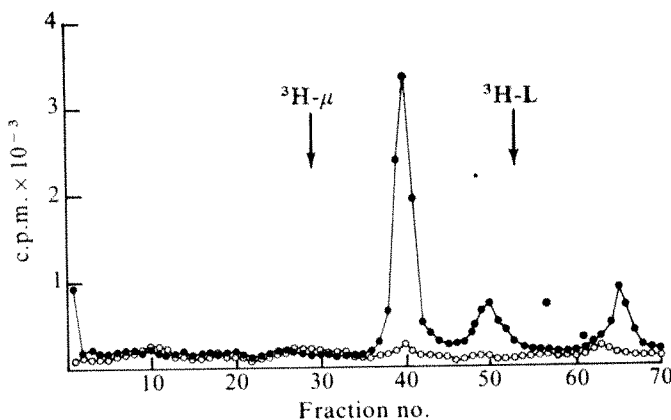
## Homology of (murine) H-2 and (human) HLA with a chicken histocompatibility antigen

THE major histocompatibility complex (MHC) is a cluster of genes controlling a variety of immunological and non-immunological phenomena<sup>1</sup>. Membrane-bound glycoprotein products of the MHC genes are of two classes: molecules of one class consist of 44,000 MW polypeptide chain which is non-covalently associated with a smaller (12,000) chain of  $\beta_2$ -microglobulin<sup>1</sup>; molecules of the second class consist of two polypeptide chains of 33,000 and 28,000

MW<sup>1</sup>. The MHC was originally discovered in the mouse<sup>2</sup>, where it is referred to as the histocompatibility-2 or H-2 system<sup>3</sup>. Later, similar systems were described in man, rhesus monkey, chimpanzee, rat, dog, rabbit and guinea pig; and, in fact, may exist in most, if not all, mammalian species<sup>4</sup>. Furthermore, clusters of genes controlling graft rejection, mixed lymphocyte reaction (MLR), immune responsiveness, and complement activity have also been reported in the chicken<sup>5</sup> and *Xenopus*<sup>6</sup>. It is tempting to speculate that these gene clusters controlling immune functions form a single evolutionary line from more primitive ancestral genes in lower vertebrates to the HLA complex in man and the H-2 complex in the mouse<sup>7</sup>. Proof of such a relationship exists for the MHC of man and mouse, where the H-2 and HLA systems show considerable amino acid sequence homology in both classes of molecules<sup>7</sup>. Because of the relative closeness of the two species (H-2 and HLA), homology is not surprising. We have investigated whether the homology extends to other vertebrate classes, such as birds: for example, is the chicken B system a true evolutionary homologue of H-2 or HLA?

Molecules isolated with the use of anti-B sera from chicken lymphocytes were used for amino acid sequence

Fig. 1 SDS-PAGE of <sup>125</sup>I-labelled B antigen precipitated from lysates of radioiodinated chicken splenocytes. Precipitates were dissolved, reduced, and electrophoresed for 16 h at 4 mA on 7.5% gels together with <sup>3</sup>H- $\mu$  and L chains. In plotting gel patterns, the <sup>3</sup>H markers were aligned. ●, Anti-B; ○, control.



				5		10		15		20		25	
CHICKEN B		HIS			ARG TYR PHE	ARG		MET			TYR		TYR
MOUSE H-2K <sup>k</sup>	PRO	HIS	SER LEU	ARG TYR PHE	HIS	THR ALA VAL	SER ARG PRO	LEU	LYS PRO ARG PHE				TYR
H-2K <sup>b</sup>	PRO	HIS	SER LEU	ARG TYR PHE	VAL	THR ALA VAL	SER ARG PRO	LEU	LYS PRO ARG TYR				TYR
HUMAN A <sub>2</sub>	GLY SER	HIS	SER MET	ARG TYR PHE	PHE	THR SER VAL	SER ARG PRO GLY		GLY GLY SER ASX	PHE ILE ALA VAL			
B <sub>7</sub>	GLY SER		SER MET	ARG TYR PHE	TYR	THR SER VAL	SER ARG PRO GLY		GLY GLU	PHE ILE	VAL		
GUINEA PIG GPLA		HIS		LEU	ARG TYR PHE	TYR	ALA VAL	PRO		PHE VAL			TYR

Fig. 2 Comparison of the N-terminal sequences of murine H-2K<sup>k</sup>, H-2K<sup>b</sup>, guinea pig GPLA, and human HLA-2 and HLA-7 with the chicken B molecule. Residues which show homologies are boxed.

analysis. White Leghorn chickens of the SC strain (Hyline SC; B<sup>2</sup>B<sup>2</sup>), 1–4 months old, were killed, spleen cell suspension prepared<sup>8</sup>, and  $0.5 \times 10^8$ – $3 \times 10^8$  cells labelled with single amino acids as described previously<sup>8</sup>. Cells were lysed in 0.5% Nonidet P40 (NP40) (Gallard Schlesinger) ( $10^8$  nucleated cells ml<sup>-1</sup>) and the lysates centrifuged and dialysed for 16–24 h at 4 °C against phosphate-buffered saline (PBS) pH 7.3. Lysates were centrifuged and the acid-precipitable radioactivity determined<sup>8</sup>. They were then treated with rabbit antisera containing antibodies against chicken Ig (RACIg). Complexes were precipitated by the addition of goat anti-rabbit Ig (GARIG). Precipitates were removed by centrifugation and the supernatants treated with anti-B serum or normal chicken serum (control) and RACIg. The anti-B serum was produced by the immunisation of B<sup>2</sup>B<sup>2</sup> chickens with B<sup>2</sup>B<sup>2</sup> cells and was extensively absorbed by red blood cells to remove non-B activity. Precipitates were washed, dissolved, and electrophoresed on 7.5% SDS-polyacrylamide gels (SDS-PAGE) at 4 mA for 16 h. Gel peaks were extracted, lyophilised, and subjected to automated sequencing on a Beckman 890C sequencer using a DMAA programme<sup>9</sup>. Samples from the sequencer were counted in Beckman Cocktail D. There was no interconversion of the radiolabels, as judged by back hydrolysis and amino acid analysis of portions of the immunoprecipitates<sup>8</sup>.

In approximately 20 separate labellings, the radioactivity in the anti-B precipitate represented 1–5% of the acid precipitable radioactivity. As shown in Fig. 1, analysis of the anti-B precipitates indicated the presence of molecules of 44,000, 26,000, and 12,000, consistent with previous studies of the chicken B antigens by Ziegler and Pink<sup>9</sup>. The 44,000 molecule presumably represents the H chain of the B antigen and the 12,000 molecule, the  $\beta_2$ M analogue. The 26,000 MW peak, which was generally small and occasionally absent may represent the Ia analogue<sup>9</sup>.

As shown in Fig. 2, eight assignments were made in the N-terminal 27 residues of the molecule. Although more than one amino acid was not found at any one position, the phenylalanine at 8 and the tyrosine at 22 remain tentative, because of the significantly lower yields at these positions compared with the others. It is possible that two distinct molecules are being sequenced (analogous to the D and K ends of H-2), only one of which has a phenylalanine at position 8 or a tyrosine at position 22.

Figure 2 also compares the partial chicken B sequence with the guinea pig GPLA<sup>10</sup>, two mouse H-2 molecules<sup>8,11–14</sup>, and two human HLA molecules<sup>15–17</sup>. Of the five residues which are identical in the first 27 residues of H-2, HLA, and GPLA, three (residues 3, 6, 7) and possibly four (residue 8) are also identical in the chicken B

sequence. Tyr at position 27 is homologous with both mouse alleles and GPLA. The only definite difference is in the Val–Met interchange at position 12. Proline, at position 15, has not been tested in the chicken. Tyr, a tentative assignment in position 22, is homologous with H-2K<sup>b</sup> and Arg, in position 9, shows no homology with the other proteins. This is not surprising as position 9 is presently the most variable position in transplantation antigens. The high degree of homology among those residues shared with the human, guinea pig, and mouse histocompatibility antigens strongly supports the notion that the B system, which diverged from the H-2-HLA precursor 75–200 Myr ago, is the evolutionary homologue of H-2, HLA, and GPLA.

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- Klein, J. in *The Biology of the Mouse Histocompatibility-2 Complex* (Springer, New York, 1975).
- Gorer, P. A. *Br. J. exp. Path.* **17**, 42–50 (1936).
- Gorer, P. A., Lyman, S. & Snell, G. D. *Proc. R. Soc. Lond.* **135**, 499–505 (1948).
- Götze, D. (ed.) *The Major Histocompatibility Complex* (Springer, New York, in the press).
- Pazderka, F., Longnecker, B. M., Law, G. R. J. & Ruth, R. F. *Immunogenetics* **2**, 101–130 (1975).
- DuPasquier, L., Chardonnens, X. & Miggianno, V. C. *Immunogenetics* **1**, 482–496 (1975).
- Klein, J. in *The Major Histocompatibility Complex* (ed. Götze, D.) (Springer, New York, in the press).
- Vitetta, E. S., Capra, J. D., Klapper, D. G., Klein, J. & Uhr, J. W. *Proc. natn. Acad. Sci. U.S.A.* **73**, 905–909 (1976).
- Ziegler, A. & Pink, R. J. *biochem. J.* **251**, 5391–5396 (1976).
- Schwartz, B. D., Kask, A. M. & Shevach, E. M. *Cold Spring Harbor Symp. quant. Biol.* **41**, 397–403 (1976).
- Silver, J. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 599–603 (1976).
- Capra, J. D., Vitetta, E. S., Klapper, D. G., Uhr, J. W. & Klein, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3661–3665 (1976).
- Ewenstein, B. M., Freed, J. H., Mole, L. E. & Nathanson, S. G. *Proc. natn. Acad. Sci. U.S.A.* **73**, 915–918 (1976).
- Henning, R., Milner, R. J., Reske, K., Cunningham, B. A. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 118–121 (1976).
- Ballou, B., McKean, D., Freedlander, E. F. & Smithies, O. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4487–4491 (1976).
- Terhorst, C., Parham, P., Mann, D. L. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 910–914 (1976).
- Appella, E. et al. *Cold Spring Harb. Symp. quant. Biol.* **41**, 341–349 (1976).



## Depurination decreases fidelity of DNA synthesis *in vitro*

DEPURINATION of DNA results from the breakage of the glycosidic bond between the purine base and the deoxyribose moiety of the purine nucleotides without disrupting the structural integrity of the phosphodiester backbone. Measurements of the rates of depurination of both synthetic polynucleotides and natural DNA in various conditions *in vitro* suggest that this damage to DNA is a frequent cellular event<sup>1</sup>. The *in vivo* rate constant has been estimated<sup>1</sup> to be of the order of  $1.8 \times 10^{-9} \text{ min}^{-1}$ . Alkylation<sup>2,3</sup> and specific glycosidases<sup>4,5</sup> increase the depurination rate constants of those altered bases to  $0.08\text{--}1.4 \times 10^{-3}$  and  $4.0 \times 10^{-3}$ , respectively. We have therefore been concerned with the possible consequences which may result during replication, repair or transcription when the template contains unrepaired apurinic sites. The biological effects of depurination are not clear. Depurination of DNA has been equated with strand breakage, a potentially lethal lesion<sup>6,7</sup>, but other evidence indicates that the apurinic site may be quite stable in cells with a half life of up to several months<sup>8,9</sup>. It is possible, therefore, that depurination of DNA can be a mutagenic event. Mutagenesis by depurination might occur either by an insertion of an incorrect nucleotide opposite the apurinic site or by a deletion at that point during DNA replication or repair. In view of the potentially large number of apurinic sites and their apparent stability, we investigated the effects of depurination of DNA on the fidelity of DNA synthesis *in vitro*. Recent reports suggest that modification of polynucleotide or DNA templates by various agents, including chemical carcinogens and ultraviolet irradiation, may affect the fidelity of DNA synthesis<sup>10-12</sup>. We report here that depurination of the synthetic polynucleotide poly d(A-T) results in a decrease in the fidelity of DNA synthesis *in vitro* using the DNA polymerase from avian myeloblastosis virus (AMV). This is the first demonstration of a possible relationship between depurination and mutagenesis through incorporation of non-complementary nucleotides during DNA synthesis *in vitro*.

The effect of depurination of poly d(A-T) on the fidelity of DNA synthesis with AMV DNA polymerase is shown in Table 1. Poly d(A-T) was depurinated by exposure to heat and acid, and the fidelity of DNA synthesis was measured by the simultaneous incorporation of the complementary ( $\alpha\text{-}^{32}\text{P}$ -dTTP) and non-complementary ( $^3\text{H}$ -dGTP) nucleotides. With the non-depurinated template, AMV DNA polymerase incorporated one molecule of non-complementary nucleotide for every 4,060 molecules of complementary nucleotide. The number of non-complementary nucleotides incorporated relative to the number of complementary nucleotides incorporated (error rate) increased in direct proportion to the extent of depurination. The error rate increased from 1/4,060 to 1/1,500 as a result of depurination (Table 1, experiment 1).

The reaction requirements for the incorporation of non-complementary nucleotide with either depurinated or non-depurinated templates are shown in Table 2. The incorporation of non-complementary nucleotide was dependent on  $\text{Mg}^{2+}$ , dTTP, enzyme and template. Elimination of any of these components markedly reduced incorporation of non-complementary nucleotide. The requirements for incorporation of non-complementary nucleotide are similar for both the depurinated and non-depurinated templates, and suggest that polymerisation is required for the incorporation of non-complementary nucleotides.

The effect of depurination on the rates of incorporation of the complementary and non-complementary nucleotides is shown in Fig. 1. The rate of incorporation of both

the complementary and non-complementary nucleotides were lower with the depurinated templates as compared with the non-depurinated template. Moreover, the rates were decreased in proportion to the extent of depurination. The coordinate incorporation of complementary and non-complementary nucleotides for each template suggests that these errors are evenly distributed during synthesis. Also, with each template, the incorporation of non-complementary nucleotide relative to the complementary nucleotide was constant with time of incubation, so the increase in error rate due to depurination was not simply the result of diminished synthesis. On the contrary, when the time of incubation was varied so that net synthesis with each modified template was the same (Table 1, experiment 2), depurination caused an absolute increase in the incorporation of non-complementary nucleotides.

The results presented here demonstrate a possible relationship between depurination of DNA and error in DNA replication. The fidelity of DNA synthesis with AMV DNA polymerase is decreased as a result of depurination of the poly d(A-T) template (Table 1, experiment 1). This mis-incorporation is also shown to be directly proportional to the extent of depurination (Table 1, experiment 2). Deletion experiment (Table 2) and kinetic studies (Fig. 1), suggest that the incorporation of non-complementary nucleotides is dependent on polymerisation and occurs randomly. Preliminary results indicate that a similar increase in mis-incorporation occurs using other depurinated templates and other DNA polymerases.

An analysis of the net mis-incorporation relative to the number of apurinic sites yields a ratio of 1 error for every

**Table 1** Fidelity of DNA synthesis with poly d(A-T) template and AMV DNA polymerase

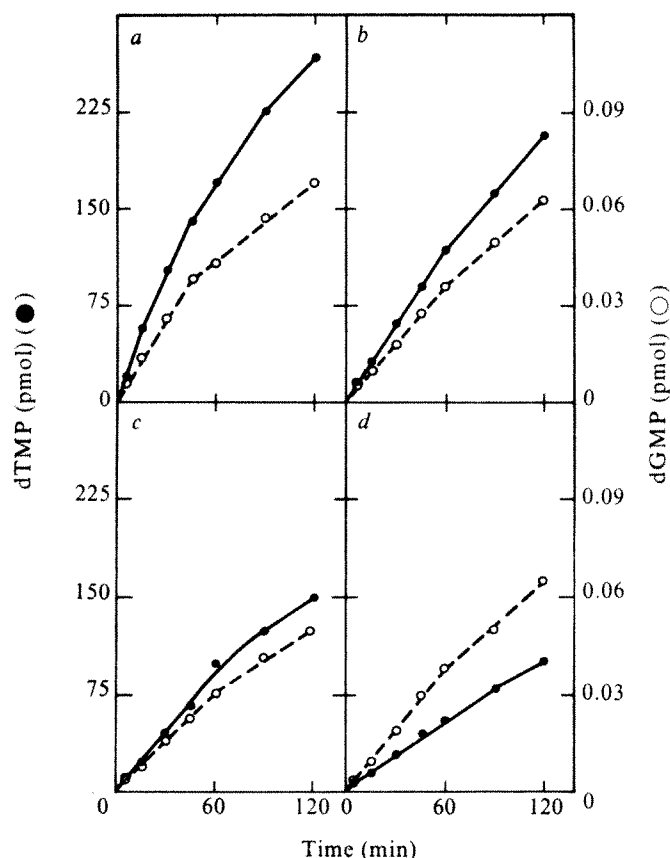
Depurination* (%)	dTMP (pmol)	dGMP (pmol)	Error rate†
Expt 1‡			
0.0	187	0.0461	1/4,060
0.6	181	0.0464	1/3,900
1.1	152	0.0419	1/3,630
1.7	130	0.0371	1/3,500
2.2	124	0.0369	1/3,360
3.4	117	0.0370	1/3,160
5.0	109	0.0375	1/2,910
6.7	94	0.0353	1/2,660
10.1	61	0.0274	1/2,220
13.4	43	0.0218	1/1,970
20.2	15	0.0100	1/1,500
Expt 2§			
0.0	100	0.0230	1/4,350
1.1	98	0.0252	1/3,890
2.2	97	0.0272	1/3,570
5.0	96	0.0323	1/2,970
10.1	102	0.0441	1/2,310

\*Depurination of  $^3\text{H}$ -dA-labelled and unlabelled poly d(A-T) templates at a concentration of  $1 \text{ mg ml}^{-1}$  was carried out at pH of 2.75 and a temperature of  $55^\circ\text{C}$ . The release of  $^3\text{H}$ -adenine was measured by thin layer chromatography with cellulose plates and solvent containing *n*-butanol, isobutyric acid, 25% ammonia and water (3.0 : 1.5 : 0.1 : 1.0). The rate of depurination was 0.1% per min. No measurable depurination or change in error rate was achieved with either heat or acid treatment alone.

†Error rate = pmol dGMP incorporated / pmol dTMP incorporated.

‡Fidelity assays were performed in duplicate, were incubated at  $37^\circ\text{C}$  for 60 min and contained (final volume  $25 \mu\text{l}$ ): 50 mM Tris-HCl, pH 7.8; 5 mM  $\text{MgCl}_2$ ; 25  $\mu\text{M}$  dATP; 25  $\mu\text{M}$   $\alpha\text{-}^{32}\text{P}$ -dTTP (20 d.p.m.  $\text{pmol}^{-1}$ ); 25  $\mu\text{M}$   $^3\text{H}$ -dGTP (50,000 d.p.m.  $\text{pmol}^{-1}$ ); 2  $\mu\text{g}$  poly d(A-T) (modified as noted in the table); and 25  $\mu\text{g}$  homogeneous AMV DNA polymerase. The reactions were terminated, washed and processed for determination of acid precipitable radioactivity as previously described<sup>13</sup>. Incorporation in the absence of incubation was 0.5 pmol dTMP and 0.0134 pmol dGMP which were subtracted from the values listed.

§Assay conditions were as described above with the exception of time of incubation which was 30 min, 40 min, 50 min, 60 min, and 120 min for the 0.0%, 1.1%, 2.2%, 5.0% and 10.1% depurinated templates, respectively.



**Fig. 1** Assay conditions were as described in the legend to Table 1 with the exception of time of incubation which was varied as shown. *a*, 0% depurination; *b*, 1.7% depurination; *c*, 5.0% depurination; *d*, 10.1% depurination. ●, Incorporation of dTMP; ○, dGMP incorporated.

500 apurinic sites. This frequency is lower than would occur if any one of the three nucleotides in the reaction mixture was incorporated randomly at each apurinic site. Several plausible explanations can account for this observation. First, there may be something unique about 1 in every 500 apurinic sites leading to misincorporation. Second, there may be a looping-out phenomena at most apurinic sites, producing deletions which we cannot monitor. Finally, factors other than hydrogen bonding between base pairs may be involved in base selection so as to select the complementary base rather than the non-complementary base. A detailed analysis of each of these and other possibilities is currently under investigation.

The increased mis-incorporation by DNA polymerases with depurinated templates *in vitro* raises the possibility that similar events may take place *in vivo* with mutagenic effects. A spontaneous depurination rate constant of

$1.8 \times 10^{-8} \text{ min}^{-1}$  although quantitatively small may be significant. If one assumes that the DNA of a mammalian cell contains  $10^9$  purine residues, this rate would produce two apurinic sites per min per cell. Alkylation in conjunction with specific glycosidases could increase this rate to more than 50 apurinic sites per min per cell. The large number of apurinic sites with miscoding potential produced by the action of chemical carcinogens, either spontaneously or in conjunction with glycosidases, may be a common mechanism of action of chemical carcinogens leading to mutations and/or carcinogenesis.

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1. Lindahl, T. & Nyberg, B. *Biochemistry* 11, 3610-3618 (1972).
2. Margison, G. P., Capps, M. J., O'Connor, P. J. & Craig, A. W. *Chem.-Biol. Interact.* 6, 119-124 (1973).
3. Margison, G. P. & O'Connor, P. J. *Biochim. biophys. Acta* 331, 349-356 (1973).
4. Kirtikar, D. M. & Goldthwait, D. A. *Proc. natn. Acad. Sci. U.S.A.* 71, 2022-2026 (1974).
5. Lindahl, T. *Nature* 259, 64-66 (1976).
6. Brookes, P. & Lawley, P. D. *Biochem. J.* 80, 496-503 (1961).
7. Brookes, P. & Lawley, P. D. *Biochem. J.* 89, 138-144 (1963).
8. Laurence, D. J. R. *Proc. R. Soc. A* 271, 520-530 (1963).
9. Lindahl, T. & Andersson, A. *Biochemistry* 11, 3618-3623 (1972).
10. Caillet-Fauquet, P., Defais, M. & Radman, M. *J. molec. Biol.* (in the press).
11. Sirover, M. A. & Loeb, L. A. *Nature* 252, 414-416 (1974).
12. Abbott, P. J. & Saffhill, R. *Nucleic Acids Res.* 4, 761-769 (1977).
13. Battula, N. & Loeb, L. A. *J. biol. Chem.* 249, 4086-4093 (1974).

## Membrane-bound carotenoid in *Micrococcus luteus* protects naphthoquinone from photodynamic action

ALTHOUGH carotenoid pigments are widely distributed in nature, their function is little understood. Studies of non-photosynthetic bacteria have led to the conclusion that these pigments are present in the plasma membrane where they protect membrane components from photo-oxidation. We report here a study of detergent-extracted carotenoid which is not protein-bound but associated with membrane lipid. The lipid-carotenoid micelles are shown to protect naphthoquinones from photodestruction, a function attributed to the native pigment.

Two major biological roles have been assigned to carotenoids in plants and prokaryotes. In photosynthetic organisms, these pigments are involved in trapping light energy<sup>1</sup>. A more general role applicable to both photosynthetic and non-photosynthetic cells, is protection from photodynamic action. Thus in photosynthetic bacteria, carotenoid-less cells, obtained by mutation or by the use of inhibitors, were killed when illuminated in aerobic but not anaerobic conditions<sup>2,3</sup>. In contrast, carotenoid-containing cells remained viable. Evidence suggested that bacteriochlorophyll acted as photosensitizer<sup>4</sup>. In non-photosynthetic bacteria early experiments showed that carotenoid-less cells were killed by light only when low concentrations of a suitable sensitising dye were added<sup>5</sup>. Subsequently, high light intensities in the absence of added sensitizer were found to kill carotenoid-less strains<sup>6</sup> while carotenoid-containing cells were afforded protection. It may be postulated that a membrane-bound pigment such as a flavoprotein or cytochrome was acting as the endogenous photosensitizer<sup>7</sup>. In *Micrococcus luteus* (*Sarcina lutea*) a study of the effects

**Table 2** Incorporation of complementary and non-complementary deoxynucleotides in various conditions with poly d(A-T) template and AMV DNA polymerase

Conditions	No depurination			13.4% Depurination		
	dTMP (pmol)	dGMP (pmol)	Error rate	dTMP (pmol)	dGMP (pmol)	Error rate
Control	113	0.0243	1/4,650	36	0.0209	1/1,720
-Mg <sup>2+</sup>	0	0.0000	—	0	0.0000	—
-Poly d(A-T)	0	0.0034	—	0	0.0034	—
-dTTP	0	0.0060	—	0	0.0062	—
-Polymerase	0	0.0000	—	0	0.0000	—

Assay conditions were as described in the legend to Table 1 with the exceptions noted.

**Table 1** Electrophoresis of the carotenoid extract on a sucrose discontinuous gradient

Fraction no.	Sucrose (g per 100 ml)	Carotenoid ( $\mu\text{g ml}^{-1}$ )	Phospholipid ( $\text{mg ml}^{-1}$ )	Protein ( $\text{mg ml}^{-1}$ )	Protein (g) per mole of carotenoid ( $\times 10^3$ )
I	21	3.5	0.46	0.02	3.4
II	21	3.4	0.41	0.03	5.3
III	21-25	1.1	0.38	0.01	5.5
IV	25	1.0	0.38	0.01	6.0
V	25	1.0	0.31	0.24	144
VI	50	0.6	0.31	0.5	500
VII	50	0.2	0.22	0.18	540
VIII	50-75	0.1	0.20	0.17	1,020
IX	75	0	0.06	0.17	—

A 15 ml sample of dialysed emulphogene extract was added to the discontinuous sucrose gradient column prepared according to the method of Hjerten<sup>19</sup> but containing an additional sucrose layer of 21% sucrose 0.56 M NaCl. Tris-citrate buffer, pH 8.6, 0.04 M was used as buffer. 240V (13 mA) were applied to the column for 20 h at 4 °C. Fractions were withdrawn from the column as shown. Nitrogen was determined by the ninhydrin method of Jacobs<sup>23</sup> and the protein content calculated assuming a 16% nitrogen content and no nitrogen contribution from phospholipid. Phospholipid was derived from the phosphorus content<sup>24</sup> assuming that the phospholipid contained 4% phosphorus. Carotenoid was obtained assuming an  $A_{1\text{cm}}^{440} = 2,500$  at 447 nm and an arbitrary molecular weight of 600.

of light on respiration in the presence of the dye toluidine blue showed that endogenous carotenoid partially protected several sites in the respiratory chain<sup>8</sup>. Using high light intensities and relying on endogenous sensitizers, carotenoid protected one photosensitive site, the respiratory quinone, menaquinone<sup>9,10</sup>.

Although several authors have suggested that the carotenoid pigment located in the plasma membrane is specifically associated with protein or lipoprotein, this has rarely been shown<sup>11,12</sup>. We have therefore attempted to isolate and purify the carotenoid pigments of *M. luteus* in a form similar to that *in vivo* using a mild non-ionic detergent, emulphogene (Mulgofer BC720). Although non-ionic detergents have been shown to remove lipid from membrane lipoproteins<sup>13</sup>, complete removal is not always achieved even at high concentrations<sup>14,15</sup>.

Overnight shake cultures grown at 28 °C on nutrient broth were used to prepare plasma membranes by mild osmotic shock after lysozyme treatment<sup>10</sup>. The membranes were extracted with 0.6% emulphogene for 35 min at room temperature which solubilised the bulk of the major carotenoids (sarcinaxanthin, sarcinaxanthin mono-D-glucoside and decaprenoxanthin<sup>16,17</sup>). The membranes themselves retained their integrity after extraction as viewed by either phase contrast or electron microscopy using negative staining (compare erythrocyte ghosts<sup>18</sup>). The extract showed a large number (at least 20) of protein bands when subjected to electrophoresis at pH 8.6 in 7.5% acrylamide gels containing 0.1% (w/v) emulphogene. The absorption maxima of the extracted carotenoid (418, 447 and 476 nm) are the same as those of the membrane-bound pigment obtained by difference spectroscopy of membranes from the wild-type and a carotenoid-less mutant. Sucrose gradient electrophoresis<sup>19</sup> of the extract at pH 8.6 for 20 h gave fractions with carotenoid, protein and phospholipid contents shown in Table 1. The carotenoid band was diffuse, low in protein

content but rich in phospholipid. On analytical acrylamide electrophoresis at pH 8.6, the carotenoid fraction (I and II, Table 1) showed a single amidoschwartz-staining protein component running independently of the carotenoid band. In electrophoretic studies, the carotenoid band seemed translucent, was anodic between pH 3.1 and 8.6 and was evidently associated with phospholipid.

Fractionation of the extract on DEAE-cellulose at pH 8.6 resulted in a discrete carotenoid fraction running substantially in front of the flavoproteins (NADH and malate dehydrogenases). The carotenoid fraction had a substantial phospholipid content (12.5  $\mu\text{g}$  per  $\mu\text{g}$  carotenoid) but was low in protein (2.5  $\mu\text{g}$  per  $\mu\text{g}$  carotenoid). Partition of the emulphogene extract in a polymer system, dextran-Ficoll-polyethyleneglycol-KCNS<sup>20</sup> resulted in the carotenoid partitioning, together with the phospholipid, in the hydrophobic polyethyleneglycol phase. This phase contained protein which migrated as a single band independently of the carotenoid on acrylamide gel electrophoresis.

Using the emulphogene extract or the carotenoid phase after polymer partition and dialysis, it has been possible to demonstrate the protection of naphthoquinone (phyloquinone, vitamin K<sub>1</sub>) by carotenoid. The quinone was added to the carotenoid preparation, briefly sonicated and assayed before and after irradiation with blue (460  $\pm$  5 nm) light. The loss of quinone was compared with that in a similar preparation from a carotenoid-less mutant of *M. luteus* (Table 2). The loss of quinone was clearly greater in the carotenoid-less preparation. Some loss of quinone in the carotenoid system is to be expected since the method of preparation did not ensure that all quinone molecules were in association with carotenoid. By contrast, addition of quinone to an ethanolic extract of the organism did not result in protection of quinone by carotenoid.

Since in our analytical studies we find carotenoid migrating with phospholipid, it is probable that in the detergent

**Table 2** Irradiation of phyloquinone (vitamin K<sub>1</sub>) by blue light

	Before irradiation ( $\mu\text{g ml}^{-1}$ )	After irradiation ( $\mu\text{g ml}^{-1}$ )	% Loss of quinone
Quinone in extract from pigmented membranes	17.5	16.9	3
Quinone in extract from carotenoid-less membranes	17.6	13.6	23

Phyloquinone in 0.1 ml ethanol was added to a carotenoid extract after partition<sup>20</sup> into a polyethyleneglycol phase. The mixture was sonicated to form a micellar system of carotenoid and quinone. The preparation was irradiated with blue light (460  $\pm$  5 nm) for 12 min at 15 W m<sup>-2</sup>, the preparation having a 1 cm light path. The quinone was assayed by the borohydride reduction method of Kroger and Dadak<sup>25</sup>.



extract the carotenoid is present in phospholipid micelles. The water-insoluble naphthoquinone is likely to be held in the micelles in relatively close proximity to the carotenoid, a situation not achieved in ethanolic solution. The protection of quinone by transfer of excitation energy to carotenoid will therefore be facilitated.

In studies of respiration, the function attributed to carotenoid is protection of the quinone<sup>10</sup>. Since the extracted carotenoid carries out the same function and has the same absorption maxima as in the membrane-bound state, we believe that the bulk of the pigment is associated with membrane lipid *in vivo*. In viability studies the protective function of carotenoid has been shown to depend not only on chromophore length<sup>21</sup> but also on concentration<sup>22</sup>. Thus the bulk carotenoid seems to be necessary for photoprotection. This does not, however, rule out the possibility of small amounts of carotenoid being protein-bound<sup>12</sup> or of the pigment being associated with lipoprotein, but such states would not be essential for the protective function. We conclude that the lipid-associated carotenoid in *M. luteus* has a role in the photoprotection of naphthoquinone.

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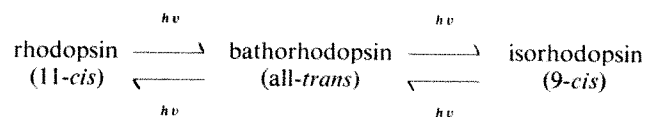
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- Goedheer, J. C. *Biochim. biophys. Acta* **172**, 252–265 (1969).
- Griffiths, M., Sistrom, W. R., Cohen-Bazire, G., Stainer, R. Y. & Calvin, M. *Nature* **176**, 1211–1215 (1955).
- Cohen-Bazire, G. & Stainer, R. Y. *Nature* **181**, 250–252 (1958).
- Sistrom, W. R., Griffiths, M. & Stainer, R. Y. *J. cell. comp. Physiol.* **48**, 473–515 (1956).
- Kunisawa, R. & Stainer, R. Y. *Arch. Mikrobiol.* **31**, 146–156 (1958).
- Mathews, M. M. & Sistrom, W. R. *Arch. Mikrobiol.* **35**, 139–146 (1960).
- Mathews, M. M. *Photochem. Photobiol.* **2**, 1–8 (1963).
- Prebble, J. & Huda, A. S. *Photochem. Photobiol.* **17**, 255–264 (1973).
- Prebble, J. & Huda, A. S. *Archiv. Microbiol.* **113**, 39–42 (1977).
- Anwar, M. & Prebble, J. *Photochem. Photobiol.* **26** (in the press).
- Thirkell, D. & Hunter, M. I. S. *J. gen. Microbiol.* **58**, 289–292 (1969).
- Thirkell, D. & Hunter, M. I. S. *J. gen. Microbiol.* **62**, 125–127 (1970).
- Aithal, H. N., Janki, R. M., Gushulak, B. D. & Tustanoff, E. R. *Arch. Biochem. Biophys.* **176**, 1–11 (1976).
- Osborne, H. B., Sardet, C. & Helenius, A. *Eur. J. Biochem.* **44**, 383–390 (1974).
- Robinson, N. C. & Capaldi, R. A. *Biochemistry* **16**, 375–381 (1977).
- Norgard, S., Francis, G. W., Jensen, A. & Liaaen-Jensen, S. *Acta chem. scand.* **24**, 1460–1462 (1970).
- Jensen, S. L., Arpin, N., Norgard, S. & Francis, G. W. *Acta chem. scand.* **27**, 2321–2334 (1973).
- Coleman, R., Holdsworth, G. & Finean, J. B. *Biochim. biophys. Acta* **436**, 38–44 (1976).
- Hjertén, S. *Biochim. biophys. Acta* **237**, 395–403 (1971).
- Albertsson, P. *Biochemistry* **12**, 2525–2530 (1973).
- Mathews-Roth, M. M. & Krinsky, N. I. *Photochem. Photobiol.* **11**, 555–557 (1970).
- Mathews-Roth, M. M. & Krinsky, N. I. *Photochem. Photobiol.* **11**, 419–428 (1970).
- Jacobs, S. *Nature* **183**, 262 (1959).
- Allen, R. J. L. *Biochem. J.* **34**, 858–865 (1940).
- Kroger, A. & Dadak, V. *Eur. J. Biochem.* **11**, 328–340 (1969).

## Temperature and wavelength effects on the photochemistry of rhodopsin, isorhodopsin, bacteriorhodopsin and their photoproducts

The primary photochemical event in visual pigments has become a matter of considerable controversy<sup>1–3</sup>. We recently reviewed the various models that have been proposed and argued that the accumulated evidence strongly favours the original suggestion of Kropf and Hubbard that the primary step involves a *cis-trans* isomerisation<sup>4</sup>. Evidence for isomerisation is based on the photoequilibrium that can be established, both at 77 K (ref. 5) and at room temperature<sup>6</sup>, between rhodopsin, bathorhodopsin (its primary photoproduct), and

isorhodopsin (which contains a 9-*cis* chromophore). The equilibrium can be represented by

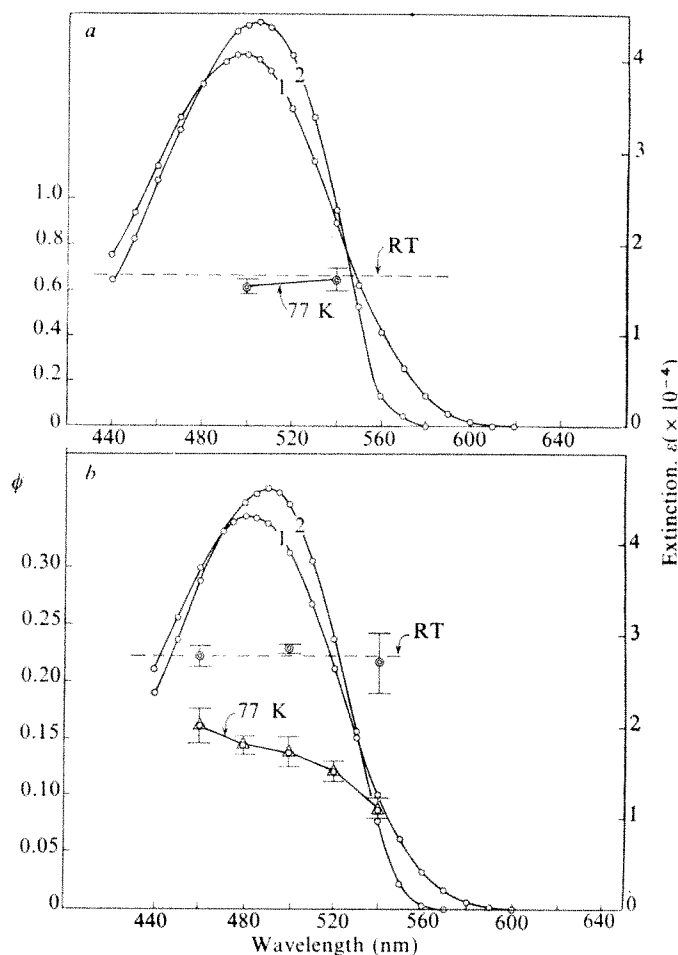


This strongly suggests that the isomeric configuration of the chromophore of the common bathorhodopsin intermediate is all-*trans* retinal, and thus light has isomerised the chromophore from 11-*cis* to all-*trans*.

It is of considerable interest to obtain a quantitative description of the physical processes involved in the primary photochemical event. Based on an analysis of the temperature and wavelength effects on the photochemistry of rhodopsin and protonated Schiff bases in solution, we proposed a model<sup>4</sup> describing a potential energy curve for the excited state of the visual pigment. The major conclusion of the model is that the protein moiety of the pigment efficiently channels the excitation energy of the chromophore into a single potential minimum along the 11–12 torsional coordinate.

We extend here our original experiments and analysis to the

**Fig. 1a** Absorption spectra of bovine rhodopsin in 67% glycerol and 2% digitonin at (1) room temperature and (2) 77 K. Corrections were made for scattering by subtracting the spectrum of the same sample bleached in the presence of hydroxylamine. Corrections were also made for a 7.7% solvent contraction at 77 K. The dashed line represents the quantum efficiency for bleaching rhodopsin at room temperature<sup>10,11,12</sup>. The circles represent the average of five measurements of the quantum efficiency at 77 K as described in the text. Standard deviations are given by the error bars. **b**, Isorhodopsin spectra under same conditions as in **a**. Isorhodopsin was prepared by regeneration of opsin with 9-*cis* retinal. Circles represent room temperature quantum efficiencies and triangles represent the quantum efficiencies at 77 K.

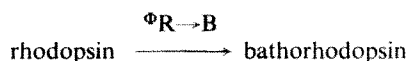


artificial pigment, isorhodopsin, and to the purple membrane protein of *Halobacterium halobium*, bacteriorhodopsin. The results for isorhodopsin provide a strong independent confirmation of the original model. Moreover, our analysis of bacteriorhodopsin photochemistry suggests a protein induced *cis-trans* isomerisation for that pigment as well.

Our experimental approach is to test the effects of temperature and excitation wavelength on the absolute yield of forming the bathorhodopsin from both rhodopsin and isorhodopsin as well as the purple membrane protein. The amount of isorhodopsin and rhodopsin converted to bathorhodopsin by a low dose irradiation at 77 K was determined by warming the sample to room temperature (where the bathorhodopsin decays to all-*trans* retinal and opsin) and measuring the amount of unbleached pigment remaining. The amount of the purple membrane protein converted to its bathorhodopsin, K, was calculated from the absorption of the mixture formed after a short irradiation using the extinction coefficients of the purple membrane and K (shown in Fig. 2). Quantum yields were calculated according to Dartnall<sup>8,9</sup> using rhodopsin as an actinometer and correcting for changes in extinction coefficient with temperature (Figs 1a and 1b). Quantum fluxes at longer wavelengths were determined with a rhodopsin calibrated photodiode.

As shown in Figs 1a and 2, we found that the quantum yield of the primary photo-event of rhodopsin and the purple membrane protein is, within experimental error, independent of excitation wavelength and temperature over a 220 K range. Moreover, Fig. 2 shows the quantum yield of the photoreversal from the bathorhodopsin K to the purple membrane pigment is also independent of temperature. On the other hand, the quantum yield for bleaching isorhodopsin (which has no wavelength dependence at room temperature) shows a significant decrease at lower temperatures (Fig. 1b). This lower quantum yield at 77 K was also wavelength dependent, the yields being smaller as wavelength increased.

The wavelength and temperature independence of the bleaching of rhodopsin strongly suggests that *cis-trans* isomerisation takes place after complete thermal relaxation and requires no activation energy. This situation may be achieved by efficiently channelling the excited system into a minimum, along a barrierless potential curve connecting the *cis* and *trans* configurations as shown in Fig. 3. This conclusion was also suggested by the observation that the quantum yields for the forward



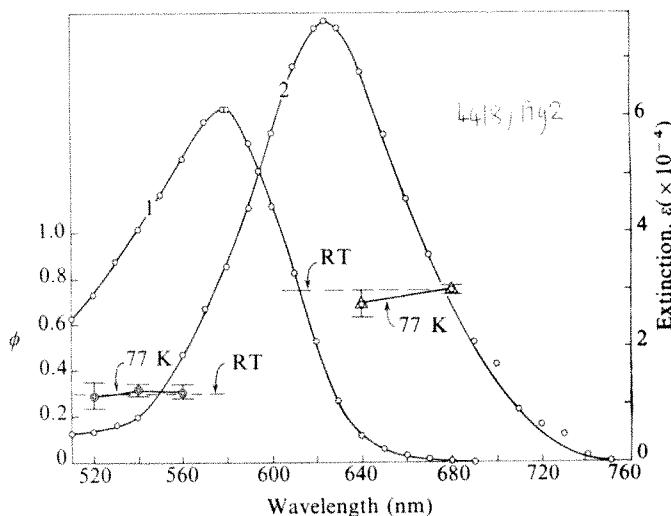
and back



reactions nearly sum to unity<sup>4</sup> which implies that most ( $\phi_1 \approx \phi_2 \approx 1$ ) excited rhodopsin and bathorhodopsin molecules populate a common point on the excited state potential surface. (This can be easily seen since  $\Phi_{R \rightarrow B} = \phi_1 \gamma_1$ ,  $\Phi_{B \rightarrow R} = \phi_2 \gamma_2$  and  $\gamma_1 + \gamma_2 = 1$ . Thus,  $\Phi_{R \rightarrow B} + \Phi_{B \rightarrow R} = 1$  only when  $\phi_1 \approx \phi_2 \approx 1$ ).

The general form of the potential curve shown on the left side of Fig. 3 is amenable to more quantitative analysis. Using the absolute and relative quantum yields at 77 K (see Fig. 3) we find  $\phi_1 = 1.0$ ,  $\phi_2 = 0.9$ ,  $\gamma_1 = 0.67$  and  $\gamma_2 = 0.33$ . Moreover, the temperature independence of the  $R \rightarrow B$  photoisomerisation shows that these values are accurate at room temperature as well (with the possible exception of  $\phi_2$  which cannot be determined at room temperature). Thus, the left side of Fig. 3 provides the first detailed characterisation of the primary photochemistry of rhodopsin.

The fact that  $\phi_2 = 0.9$  (which requires that  $\Phi_{R \rightarrow B} + \Phi_{B \rightarrow R}$  be slightly less than unity) is due to the leakage ( $\phi_3$ ) of bathorho-



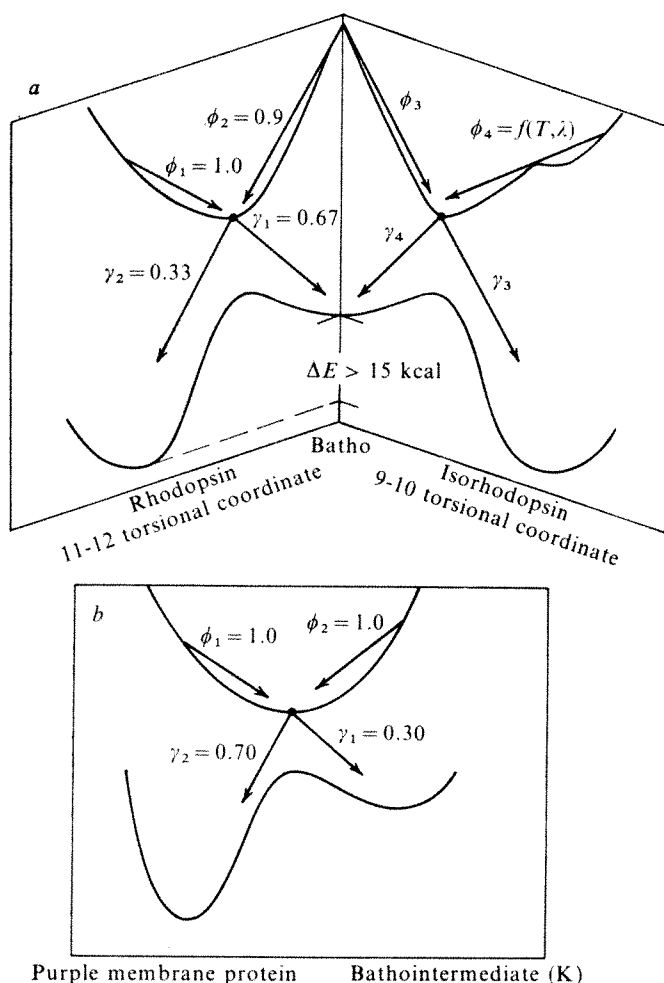
**Fig. 2** Absorption spectra at 77 K in 67% glycerol of (1) the purple membrane protein and (2) its primary photoproduct, the K intermediate. The K spectrum was determined from the spectrum of the 500 nm photosteady-state mixture, assuming it to be 28%K. This was determined by warming the mixture under conditions<sup>9</sup> which allow the complete conversion of K to M(412), revealing the percentage of pigment unconverted in the photosteady-state mixture. The circles represent the purple membrane protein quantum efficiency and the triangles represent the K quantum efficiency both at 77 K. The dashed lines represent the corresponding quantum efficiencies for the pigment (at  $-40^\circ\text{C}$  (ref. 9); a similar value is seen at room temperature)<sup>14</sup> and for the bathorhodopsin (at room temperature RT).

dopsin to the 9–10 torsional coordinate leading to the formation of isorhodopsin. An independent check of the numbers given above requires that an analysis of the  $B \rightleftharpoons I$  interconversion yield  $\phi_3 = 1 - \phi_2 \approx 0.1$ . Unfortunately, the temperature dependence of  $\Phi_{I \rightarrow B}$  and its wavelength dependence at 77 K (due perhaps to a small barrier along its potential surface) preclude an accurate determination of  $\phi_3$ ,  $\phi_4$ ,  $\gamma_3$  and  $\gamma_4$ . It is possible, however, to use the values of  $\Phi_{I \rightarrow B} = \phi_4 \gamma_4$  and  $\Phi_{B \rightarrow I} = \phi_3 \gamma_3$  to estimate  $\phi_3$ .

Taking an average value of  $\Phi_{I \rightarrow B} \approx 0.13$  (Fig. 1b) and the ratio  $\Phi_{B \rightarrow I} / \Phi_{I \rightarrow B} = 0.4$  determined previously<sup>4</sup> we find  $\phi_3 \gamma_3 = 0.05$ . (Thus our model yields  $\Phi_{B \rightarrow R} / \Phi_{B \rightarrow I} = \phi_2 \gamma_2 / \phi_3 \gamma_3 \approx 5$ , in complete agreement with the estimate of Yoshizawa and Wald<sup>5</sup>.) Simple numerical considerations (see Fig. 3a) now lead to the conclusion that  $\phi_3$  must be approximately 0.1, thus providing an important consistence check for the analysis of the  $B \rightleftharpoons R$  interconversion.

The most striking implication of the above analysis is the complete channelling ( $\Phi_1 = 1.0$ ) into the common minimum from excited rhodopsin molecules (11-*cis* configuration) and almost complete channelling of bathorhodopsin (all-*trans*) ( $\Phi_2 = 0.9$ ) to the 11–12 torsional coordinate accounting for the relationship  $\Phi_{R \rightarrow B} + \Phi_{B \rightarrow R} \approx 1$ . As can be seen from Fig. 2, the same relationship ( $\Phi_{PMP \rightarrow K} + \Phi_{K \rightarrow PMP} = 0.28 + 0.72 = 1$ ) also characterises the primary photoevent in light-adapted bacteriorhodopsin, both at 77 K and at room temperature (Fig. 2 and refs 9, 13, 14). Moreover, the photoreversibility of the various intermediates so characteristic of the bleaching sequence of visual pigments<sup>5</sup> is also a feature not only of the K intermediate but also of the 'M' (412 nm) intermediate of the purple membrane protein<sup>10</sup>. These striking photochemical analogies very strongly suggest a *cis-trans* geometry change as the primary photochemical step in bacteriorhodopsin.

The extremely simple photochemical behaviour of both rhodopsin and purple membrane protein should be contrasted with the complex patterns that characterise the photoisomerisation of retinal analogues<sup>15</sup> and other well studied systems such



**Fig. 3a** Potential energy diagrams for the rhodopsin, bathorhodopsin, isorhodopsin interconversions. The quantum efficiency for rhodopsin isomerisation is  $\phi_{R \rightarrow B} = \phi_1 \gamma_1$ , where  $\phi_1$  is the probability of reaching the bottom of the potential well of the excited state and  $\gamma_1$  is the probability of going to bathorhodopsin from the bottom of the well. Similar relations hold for  $\phi_{B \rightarrow R}$ ,  $\phi_{B \rightarrow I}$ , and  $\phi_{I \rightarrow B}$ . Quantum yields for the 11-12 coordinate are calculated from the 77 K data presented here and using  $\phi_{R \rightarrow B}/\phi_{B \rightarrow R} = 2.2$  (ref. 4). For the 9-10 coordinate, our room and low temperature data set limits for  $\phi_4$  between 0.22 and 1.0 leading to  $0.22 < \gamma_4 < 0.59$ ,  $0.41 < \gamma_3 < 0.78$  and then using  $\phi_3 \gamma_3 = 0.06$  we find  $0.07 < \phi_3 < 0.14$ . Thus, all values of  $\phi_3$  are consistent with  $\phi_2 \approx 0.9$ . **b**, Hypothetical energy level diagram for the purple membrane protein and its batho-product K. This diagram ignores the observed fluorescence which is thought to come from another excited state (ref. 19).

as stilbenes<sup>16</sup>. In contrast to the pigments, the quantum yields of the model compounds are strongly wavelength-dependent and can be 1-2 orders of magnitude less than those of the pigments. Thus, it seems that the protein facilitates isomerisation in the chromophore by altering the excited state potential energy surfaces and/or by modifying rates of radiationless deactivation. It is interesting in this context that the artificial pigment isorhodopsin seems to undergo a less efficient and more complex photochemistry than does rhodopsin.

The protein can also influence the ground state energy surfaces. Although free 11-*cis* and all-*trans* retinal have very similar free energies<sup>17</sup>, the opsin modifies the equilibrium conformation of the chromophore so that rhodopsin is at least 10 kcal mol<sup>-1</sup> lower in free energy than the final products, opsin and free all-*trans* retinal. Moreover, bathorhodopsin decays spontaneously to opsin + all-*trans* retinal and, thus, must be still higher in free energy than these products; this free energy must be obtained from the photon absorbed by

rhodopsin. A hypothetical ground state energy diagram is also included in Fig. 3.

The bathoprotect of the purple membrane protein must also contain a significant fraction of the absorbed photon's energy since the spontaneous decay of this photoproduct back to the original pigment is used to power a proton pump across the cell membrane<sup>18</sup>. In view of the basic similarity of this pigment and rhodopsin with respect to the chromophore structure and binding and their primary photochemistry, it is reasonable that similar energy storage mechanisms are operative in both pigments.

Recently, Peters *et al.*<sup>20</sup> have observed a deuterium-dependent decay of a transient species following picosecond stimulation of rhodopsin; they have proposed that light does not isomerise the chromophore, but rather initiates a proton transfer to it (see also ref. 3). We do not, however, believe that their data in any way rules out *cis-trans* isomerisation; indeed many interpretations of the data, other than the one they give, are possible. For example, the transient species may be due to a time-dependent shift in the absorption spectrum of bathorhodopsin, due to a relaxation of the protein/chromophore following isomerisation; that is, it could arise if the proton of the protonated Schiff base changes its orientation with respect to its counter ion or even switches its counter ion as a result of *cis-trans* isomerisation. This could account for the temperature and deuterium effects of the rate of formation of bathorhodopsin. In any case, the models Peters *et al.* propose for the primary event are incompatible with the known properties of bathorhodopsin discussed above and in ref. 4. Moreover, it is difficult to see how proton tunnelling as the only primary event would result in a stable species (at 77 K) which would not revert back to rhodopsin. Finally, Green *et al.*<sup>21</sup> have recently provided evidence that isomerisation in rhodopsin and isorhodopsin can occur in picoseconds at room temperature.

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- Kropf, A. & Hubbard, R., *Ann. N.Y. Acad. Sci.* **74**, 266-280 (1958).
- Huppert, D., Rentzepis, P. M. & Klier, D. S. *Photochem. Photobiol.* **25**, 193-197 (1977).
- Fransen, M. R., Luyten, W. C. M., Van Thuijl, J., Lugtenberg, J., Jansen, P. A. A., Van Brengel, P. J. G. M. & Daemen, F. J. M. *Nature* **260**, 726-727 (1976).
- Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J. & Ebrey, T. *Pure appl. Chem.* **49**, 341-351 (1977).
- Yoshizawa, T. & Wald, G. *Nature* **197**, 1279-1286 (1963).
- Rosenfeld, T., Goldschmidt, C. R. & Ottolenghi, M. *Nature* **263**, 169-171 (1976).
- Lozier, R., Bogomoloi, R. & Stoekenius, W. *Biophys. J.* **15**, 955-960 (1975).
- Dartnall, H. J. A., Goodeve, C. F. & Lythgoe, R. V. *Proc. R. Soc. A* **156**, 158-170 (1936).
- Becher, B. & Ebrey, T. *Biophys. J.* **17**, 185-191 (1977).
- Dartnall, H. J. A. *Handbook of Sensory Physiology*, **7**, (1), 122-145 (1972).
- Collins, F., Love, R. & Morton, R. *Biochem. J.* **51**, 242-248 (1952).
- Alpern, M. & Pugh, E. J. *J. Physiol., Lond.* **237**, 341-362 (1974).
- Goldschmidt, C. R., Kalisky, O., Rosenfeld, T. & Ottolenghi, M. *Biophys. J.* **17**, 179-183 (1977).
- Goldschmidt, C. R., Ottolenghi, M. & Korenstein, R. *Biophys. J.* **16**, 839-843 (1976).
- Rosenfeld, T., Alchalel, A. & Ottolenghi, M. *Proc. Lisbon Conference on Excited States of Biological Molecules* (ed. Birks, J. B.) 540-554 (Wiley, London, 1976).
- Saltiel, J., D'Agostino, J., Megarath, E. D., Metts, L., Neuberger, K. R., Weignton, M. & Zafiriou, O. C. *Org. Photochem.* **3**, 1-113 (1973).
- Hubbard, R. J. *biol. Chem.* **241**, 1814-1818 (1966).
- Oesterholt, D. & Stoekenius, W. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2853-2857 (1973).
- Alfano, R., Yu, W., Govindjee, R., Becher, B. & Ebrey, T. *Biophys. J.* **16**, 541-545 (1976).
- Peters, K., Applebury, M. & Rentzepis, P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3119-3123 (1977).
- Green, B., Monger, T., Alfano, R., Aton, B. & Callender, R. *Nature* **269**, 179-180 (1977).



# matters arising

## Membrane initiation of DNA synthesis

HOBART *et al.*<sup>1</sup> reported convincing autoradiographic evidence that DNA replication is initiated near or on the nuclear membrane in sea urchins. In their discussion of the difference between their results and those reported by others for mammalian cells<sup>2-4</sup>, they raise the possibility that the latter results might arise from repair synthesis because, "... autoradiographic analysis cannot distinguish between replication and repair synthesis of DNA ..." and "Such a situation would confuse the interpretation of autoradiographs in determining sub-nuclear sites of replication".

It is only remotely possible that this explanation is correct. DNA repair synthesis in undamaged mammalian cells is not detectable by even very sensitive methods<sup>5</sup>, let alone autoradiography. In cells whose DNA molecules are severely damaged, repair synthesis (excision) never exceeds about 1% of the amount of semiconservative synthesis that would occur simultaneously in undamaged cells<sup>6,7</sup>; even this amount of repair synthesis would not be detectable by the electron microscope autoradiographic method used by Hobart *et al.*<sup>1</sup>.

Thus, the explanation for the results showing a difference between sites of initiation of DNA synthesis in sea urchins<sup>1</sup> and in mammalian cells<sup>2-4</sup> is very likely other than the one proposed by Hobart *et al.*<sup>1</sup>.

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- Hobart, P., Duncan, R. & Infante, A. A. *Nature* **267**, 542-544 (1977).
- Williams, C. A. & Ockey, C. H. *Expl Cell Res.* **63**, 365-372 (1970).
- Huberman, J., Tsai, A. & Deich, R. *Nature* **241**, 32-36 (1973).
- Wise, G. E. & Prescott, D. M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 714-717 (1973).
- Gautschi, J. R., Young, B. R. & Painter, R. B. *Biochim. biophys. Acta* **281**, 324-328 (1972).
- Painter, R. B. *Curr. Top. Radiat. Res. Qrtly* **7**, 45-70 (1970).
- Cleaver, J. E., Thomas, G. H., Trosko, J. E. & Lett, J. T. *Expl Cell Res.* **74**, 67-80 (1972).

HOBART *et al.* REPLY: We agree with Painter and Cleaver that it seems only remotely possible that repair synthesis

of DNA could account completely for the differences between our results with sea urchins and those of others using mammalian cells. But, it remains to be determined if repair synthesis is indeed a minor activity in cells which have been maintained for prolonged periods of time in tissue culture and have been treated with various metabolic inhibitors to achieve synchronisation. In any case the results with sea urchin embryos, which are normally synchronous and in which semiconservative replication is undoubtedly the principle activity, indicate that DNA synthesis occurs on or near the nuclear membrane.

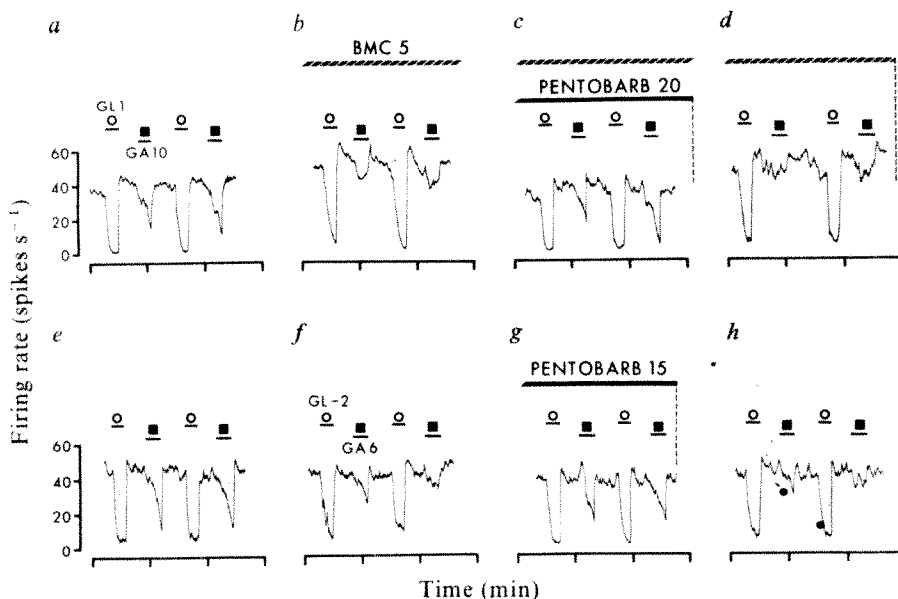
## Pentobarbitone enhancement of the inhibitory action of GABA

BOWERY and Dray<sup>1</sup> have suggested that pentobarbitone reverses the effects of the  $\gamma$ -aminobutyric acid (GABA) antagonist bicuculline methochloride (BMC), on both the superfused rat

superior cervical ganglion and rat medullary neurones *in vivo*, without potentiating the action of GABA on these tissues in the absence of BMC. Such an action does not readily account for the accentuation by pentobarbitone of inhibitions mediated by GABA in the mammalian central nervous system<sup>2</sup>, except by antagonism of an as yet undetected endogenous GABA antagonist.

We have investigated the action of pentobarbitone on 31 dorsal horn interneurons and three Renshaw cells of eight low spinal cats anaesthetised i.p. with either  $\alpha$ -chloralose and urethane (40 and 400-800 mg per kg), diallyl barbituric acid and urethane (60 and 600 mg per kg) or pentobarbitone sodium (35 mg per kg). Amino acids, acetylcholine, and pentobarbitone were administered electrophoretically from solutions in the outer barrels of seven-barrel micropipettes, the 3.6 M NaCl-containing centre barrels of which were used to record extracellular action potentials of single neurones. The following solutions were

Fig. 1 Ratemeter records of the firing of a dorsal horn interneurone in a cat anaesthetised with  $\alpha$ -chloralose and urethane. The electrophoretic ejection of glycine (GL) and GABA (GA) are indicated by the appropriate symbols, horizontal black lines and currents (nA). *b* and *c* were recorded 5 and 10 min after the commencement of the ejection of BMC which continued for 15 min, ceasing after *d*, as indicated by the broken vertical line. The ejection of pentobarbitone (PENTOBARB) commenced 3 min before *c* and again 2 min before *g*, and was also terminated at the vertical broken lines. Records *e* and *h* were 1 min after *d* and *g* respectively. Ordinates, firing rate, spikes  $s^{-1}$ , abscissae, time in min.



used: GABA (0.1, 0.2 M; pH 3), glycine (0.5 M; pH 3), L-glutamate (0.5 M; pH 7.5), DL-homocysteate (DLH, 0.2 M; pH 7.5), acetylcholine hydrobromide (ACh, 0.25 M), BMC (10 mM in 165 mM NaCl) and sodium pentobarbitone (20 mM; pH 9.5 or 250 mM in a 7 : 2 : 1 mixture by volume of water, propylene glycol and ethanol).

In confirmation of Bowery and Dray<sup>1</sup>, pentobarbitone (10–60 nA) partially reversed the antagonism by BMC of the inhibition of cell firing by GABA. With most neurones, however, in the absence of BMC, pentobarbitone ejected with the same or even lower electrophoretic currents enhanced sub-maximal inhibition of firing by GABA. Furthermore, the inhibitory action of electrophoretic glycine on many cells was also enhanced, but not to the same extent as that of GABA.

Results from one neurone are illustrated by the records in Fig. 1 of the rate of firing of a dorsal horn interneurone maintained by the continuous ejection of DLH, 8 nA. Firing was inhibited by glycine and GABA, ejected consecutively each for 7 s at fixed time intervals (Fig. 1a). During the ejection of BMC, the effect of GABA was reduced (Fig. 1b), but was restored to near control values during the simultaneous administration of pentobarbitone from an aqueous solution (Fig. 1c). This effect of pentobarbitone was reversible (Fig. 1d), as was the antagonism of GABA by BMC (Fig. 1e). Several minutes later, when the currents ejecting glycine and GABA had been reduced, in particular that of GABA to reproduce the inhibitory action observed during the ejection of BMC (compare Figs 1b, d and f), pentobarbitone also reversibly enhanced the effectiveness of GABA (Fig. 1g and h). The degree of enhancement of the GABA effect by pentobarbitone in the absence of BMC was very similar to that during the ejection of BMC.

Effects such as these, which were observed with spontaneously active cells as well as with those in which firing was maintained with DLH, glutamate or ACh, and irrespective of the anaesthetic used or whether BMC was in one of the barrels of the micro-pipettes, render unnecessary a postulate that pentobarbitone displaces BMC from receptors on central neurones<sup>1</sup>. At least in the spinal cord of the cat the increased effectiveness of GABA by pentobarbitone probably is adequate to account for the reversal of antagonism by BMC. Further experimentation will be required to determine whether this action of pentobarbitone results from interference with the cellular uptake of amino acids or from a more direct effect at postsynaptic receptors<sup>3</sup>. The importance of this latter type of action

is suggested by the frequent observation that electrophoretic pentobarbitone depressed the firing rate of neurones, and that pentobarbitone, like GABA, depolarises the terminals of group Ia afferent fibres in the cat cord (our unpublished work).

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1. Bowery, N. G. & Dray, A. *Nature* **264**, 276–278 (1976).
2. Nicoll, R. A. *Nature* **258**, 625–627 (1975).
3. Ransom, B. R. & Barker, J. L. *Brain Res.* **114**, 530–535 (1976).

**BOWERY and DRAY reply**—The results of Lodge and Curtis are most interesting but, whilst we do not dispute that enhancement of responses to GABA and glycine can occur in the absence of bicuculline methochloride (BMC), in our experiment in the rat medulla we saw no clear enhancement of sub-maximal responses to GABA or glycine using expelling currents of pentobarbitone which on the same cells reversed the antagonism produced by BMC or strychnine. Pentobarbitone in all cases did not decrease the background firing rate of the cell, an observation which differs from that of Lodge and Curtis. Only when we increased the ejecting current by 2–3-fold did pentobarbitone decrease the firing rate<sup>1</sup>. It remains to be seen whether this decrease in firing results from a GABA-mimetic effect at post-synaptic receptors. It is interesting to note that the increase in firing rate produced by BMC in the Lodge and Curtis experiment (shown in their Fig. 1b) seemed to be antagonised by the ejection of pentobarbitone (c).

Although Lodge and Curtis suggest that the enhancement of responses to

GABA could account for the observed BMC reversal on dorsal horn interneurones in the cat spinal cord we believe that under our experimental conditions on spontaneously-active neurones in the rat medulla, these phenomena are separable. Some evidence in support of this comes from results we have obtained with other drugs in the superior cervical ganglion. Although the barbiturates were the most effective in reversing BMC antagonism other central depressant drugs with quite different chemical structures, for example, benzodiazepines, amitriptyline and promethazine also reduced BMC antagonism. None of these substances significantly potentiated the effect of GABA at the concentrations employed nor did they exhibit any direct GABA-mimetic activity even at >10-fold higher doses.

Experiments with nipecotic acid in the ganglion indicate that BMC reversal by pentobarbitone is probably unrelated to any inhibition of the cellular uptake of GABA. Nipecotic acid inhibits GABA uptake in this tissue and thus potentiates the response to GABA<sup>2</sup>. Unlike pentobarbitone, however, nipecotic acid will neither prevent nor partially reverse BMC antagonism and moreover will not prevent pentobarbitone reversing the action of BMC.

Two phenomena associated with the action of pentobarbitone in relation to GABA and glycine receptors have been described—first, an enhancement of the action of GABA or glycine as reported by Lodge and Curtis and others<sup>3,4</sup> and second, a direct GABA-mimetic action as described by Nicoll<sup>4</sup>. Our results suggest that a third phenomenon may occur, that of reversal of the action of convulsants which antagonise responses to GABA and glycine.

Although separation of these phenomena may not be easy in some systems our results indicate that it is possible in the rat medulla and sympathetic ganglion and may depend on the concentration of pentobarbitone employed.

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1. Bowery, N. G. & Dray, A. *Nature* **264**, 276–278 (1976).
2. Brown, D. A. & Galvan, M. Br. *J. Pharmac.* **59**, 373–378 (1977).
3. Ransom, B. R. & Barker, J. L. *Brain Res.* **114**, 530–535 (1976).
4. Nicoll, R. A. *Brain Res.* **96**, 119–123 (1975).

## Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

# reviews

## Best kind of Natural History

S. R. J. Woodell

*Population Biology of Plants.* By John L. Harper. Pp. xxiv+892. (Academic: London, New York and San Francisco, 1977.) £30; \$58.60.

As Professor Harper states in his preface, population biology was for almost half a century the almost exclusive preserve of zoologists. In the early part of this century, Sukatschew, Tansley and especially Clements experimented on plant populations, but thereafter most plant ecologists turned to other aspects of the subject. This might seem strange. Plants are immobile. They do not have to be captured, marked and recaptured, but whereas animals respond to environmental changes by changing numbers, plants may change in either number or size, or both. It is perhaps this plasticity that has deterred some botanists from studying their populations. Numbers can be counted, size has to be measured, usually destructively.

Harper was fortunate to come into contact early in his career with Charles Elton and his group in Oxford, and then with agronomists in Aberystwyth, to whom experimentation on plant populations was nothing new. Unlike most botanists he realised the relevance of animal population studies to plants, and acted upon it. During the past twenty-five years he, his students and co-workers have done more than anyone to set the science of plant population biology on its feet, and it is appropriate that he should write the first major book about it.

A short introductory chapter on "Experiments, Analogies and Models" sets the scene, and is followed by five major sections: on "Dispersal, Dormancy and Recruitment"; "The Effects of Neighbours"; "The Effects of Predators"; "The Natural Dynamics of Plant Populations"; and finally, "Plants, Vegetation and Evolution".

Each is complete in itself, but they are all linked by the evolutionary theme that pervades the whole book. Harper, in acknowledging his debt to Darwin, states that he thinks the third chapter of the *Origin of Species* is the best ecological text ever written. Though not all would agree with this, there is no doubt that it shows an insight into interactions between organisms that has not yet been surpassed, for all that it was written 120 years ago. Harper comments (p492): "in ecology . . . the idea of evolution as a continually operating force in ecological interactions seems to be accepted unwillingly." Sadly, this is true of many, though not by any means all ecologists; such workers as G. E. Hutchinson are outstanding exceptions. This book puts the relationship between ecology and evolution firmly back into the centre of the stage.

Every chapter in Harper's book has something to say about how plant populations interact with their total environment. With animal ecologists in mind as readers, he is constantly pointing to ways in which animal and plant populations are similar and different. Apart from the plasticity of plants, two especially interesting points he makes are that the food of green plants does not reproduce and is not the result of a reproductive process (this fundamentally affects their population biology); and that plants consist of "populations of parts" (and this cannot be said of animals except perhaps corals, and some colonial Hymenoptera).

Everyone will find much of interest here; there are ideas enough to keep several workers busy for a lifetime. If I may pick chapters which especially interested me, I would select, chapter 11, "Mechanisms of Interaction Between Species", as a most stimulating, critical and thought-provoking discussion of the problems and pitfalls

inherent in studying plant interactions. The several chapters on "The Effects of Predators" comprise an account in depth of some aspects of plant-animal interactions, a fashionable field these days after long neglect. The final section, where evolution is brought to the fore, is a fascinating development in evolutionary terms of much that has gone before.

It will be clear that I liked this book; indeed, I found it difficult to put down once I had begun it. There are small irritations. In places, it reads rather like a catalogue of experiments, though every experiment described is very relevant. Those familiar with Harper's work will be familiar with much of the content of the book, but to have it all together is very valuable; and a glance at the bibliography reveals the breadth of the literature surveyed here. Harper is not quite consistent in his acceptance in some places, and rejection in others, of anecdotal evidence. These are minor flaws. A credit is the inclusion of good chapter summaries at the beginning of the book.

'Natural History' is a much-abused term, probably largely as a result of the flood of poor quality writings on it during the last century. Yet who would deny that *The Natural History of Selborne* (Gilbert White) is a great book, or that although much of what Darwin wrote was Natural History, he was a great scientist. The Oxford English Dictionary defines Natural History thus: "A work dealing with the properties of natural objects, plants or animals; a scientific account of any subject on similar lines." In those terms, I am sure that Professor Harper will not object to his book being described as an example of the best kind of Natural History. □

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## Polymer science

*Macromolecules*. Vol. 1: Structure and Properties. Pp. 532. Vol. 2: Synthesis and Materials. By Hans-Georg Elias. Pp. 131. (Wiley: London; Plenum: New York, 1977.) £24; \$42.50 each volume.

In his preface, the author describes these two volumes as a textbook, treating "the whole field of macromolecular science, from its chemistry and physics to its applications, in a not too elementary manner." To attempt this is a stupendous undertaking for a single author, but it can be said at once that the text amply justifies the claim. The original version (1971) was in German and the present text is a translation from a 1975 revised German edition—but shows no internal evidence of not having been written in English from the start.

The method adopted is that of straightforward exposition; where differing viewpoints might be held, the author usually takes a clear line, no doubt with the aim of avoiding confusion for the student. The area to be covered is vast, and within the scope of 1,100 pages most topics can receive no more than outline treatment. To enable the student to supplement this, a reading list of books and review articles is provided for each section, but there are no references to the original literature (except for historical introduction). The opportunity has evidently been taken in revision and again in translation to add recent titles to the original reading lists.

The contents of the two volumes can be described roughly as physics and chemistry, respectively; and although the work is planned as a whole, the two parts could each stand alone. Volume 1 deals with the theoretical and experimental basis of our knowledge of polymer structure, and physical properties. It is divided into three parts: structure, solution properties, and solid-state properties. Little is assumed in the way of background knowledge: chapter 5 opens with an account of X-ray scattering; and chapter 9.5 with a presentation of the basic principles of light scattering. Experimental methods are outlined, and although equipment is not described in detail, key features are frequently pointed out. Although this volume is largely concerned with principles, it is sufficiently wide-ranging to include reference to such practical matters as adhesives and glues, and the testing of insulators for resistance to tracking.

Volume 2 divides into two roughly equal parts dealing, respectively, with principles of polymerisation and individual polymers. Following an introductory chapter on general principles, the first part deals successively with polycondensations; ionic, insertion and radical

reactions, copolymerisation; and finally with reactions of macromolecules (including aging). This is a wide-sweeping survey at a very well chosen level.

The last section of the book is entitled "Polymers" and is divided into nine chapters, of which the first four deal broadly with synthetic organic polymers classified according to their main chains: carbon, carbon-oxygen, carbon-sulphur, carbon-nitrogen. The chapters are further broken down by polymer type into 25 sections, many of them further subdivided. The general method of treatment for each polymer includes monomer synthesis, polymerisation methods and mechanisms, polymer properties and reactions; all done very competently and succinctly. Final chapters extend the scope to biopolymers and inorganic chains, and serve at least to remind the student of the wide ramifications of macromolecules in nature.

Nothing could be easier for the reviewer of a work of this kind than to select a field in which he is personally expert and

to point out shortcomings and possible errors of judgement. Inevitably, when an author undertakes to review such a vast field he will succeed better with some areas than others. What is more pertinent to ask is (a) whether the work succeeds in its declared aim, and (b) what other needs it might serve. On the first of these counts, there is no doubt that any student who works systematically through this text will acquire a broad knowledge and understanding of polymer science and its applications. Spread over the three years of a Ph.D. course, it would provide a superb accompaniment to the experimental study of a limited topic. For the reviewer, its role will be that of an admirable first source of information, providing a coherently argued introduction and a guide to further reading. On both counts, it is a notable addition to polymer literature.

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## Volcanic leitmotive

*Carbonatite-Nephelinite Volcanism*. By Michael J. Le Bas. Pp. 347. (Wiley: London, New York and Toronto, 1977.) £22; \$44.

THERE is a long standing joke that one can doze off during one of Wagner's *Ring of the Nibelungen* operas, only to wake up ten minutes later to find the same singer standing on the same spot singing the same song. One gets the same impression from Le Bas' book. No matter where one dips into it, there is a certain sameness about the text. Tortuous rock names like uncomphagrite, turjaite, melteigite, ijolite and urtite keep cropping up in the prose like Wagnerian leitmotive. To appreciate the *Ring*, one has to be able to recognise the leitmotive. The same is true of this book: without a firm grasp of the convoluted terminology of hyper-alkalic volcanism, one is lost.

The first sentence of the first paragraph of the text starts: "The classic igneous intrusions of Fen (Brogger, 1921; Saether, 1957) and Alno (Eckermann, 1948) in Scandinavia, of Magnet Cove, Arkansas (Erickson and Blade, 1963), of Iron Hill, Powderhorn, Colorado . . .". This bleak style is relentlessly preserved throughout the 24 chapters of the book. Nineteen of these are detailed descriptions of individual areas or volcanoes in East Africa written by Le Bas and his colleagues who worked in the area

from 1963 to 1969. Many of these chapters are based on the distilled and condensed Ph.D. theses of research students.

Of most interest to this reviewer was the third chapter, in which Le Bas unravels the nomenclature of the rock suites—this is essential reading and should come before chapter 1—and the last two, which cover magmatic and metasomatic processes and the petrogenesis of the carbonatite nephelinite suite. There are two appendices, containing geochronological and major element geochemical data; there are few trace element data.

Le Bas has produced a thoroughly scholarly work summarising many years of work by him and his colleagues in East Africa, and one which is unequivocally aimed at a specialised readership. The descriptive chapters will undoubtedly provide a valuable source of information to workers studying alkalic volcanism in East Africa and it is clearly useful to have all this data published under one cover. It is a pity, however, that Le Bas did not broaden his scope slightly. There must be many potential readers, not directly involved in the field, who would be glad of an up-to-date and intelligible review of the fascinating petrological problems posed by the carbonatite-nephelinite suites. Only a small proportion of this book is likely to interest them.

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## Molecular trees

*Molecular Anthropology: Genes and Proteins in the Evolutionary Ascent of the Primates.* Edited by M. Goodman, R. E. Tashian and J. H. Tashian. Pp. 466 (Plenum: New York and London, 1977.) \$42.

THIS volume contains 20 chapters, most of which were presented at a symposium in Austria during July 1975. The chapters are grouped under five headings: "Background to Some Key Issues"; "Molecular Evolution as Interpreted by Mathematical Models"; "Primate Phylogeny and the Molecular Clock Controversy"; "Primate Evolution Inferred from Amino Acid Sequence Data"; and "Multigene Families and Genetic Regulation in the Evolution of Man". Forty-nine authors contributed to the compilation, which provides an excellent interplay between scientists in various fields, especially biochemistry, anthropology and genetics.

There is a chapter by E. L. Simons, a palaeontologist, on the fossil record of primate phylogeny, and one on splitting times among hominoids as deduced from the fossil record, by Alan Walker. These provide an interesting and necessary counterpoint for a host of molecular and biochemical phylogenetic trees that are constructed, by authors of other chapters, on the basis of differences between homologous proteins. Various monkeys and other vertebrates have kindly furnished the proteins, especially globins, whose amino acid sequences and immunological cross-relationships are used for the construction of these trees. There is also a short chapter on satellite DNA and ribosomal genes in primates. G. W. Lasker gives us his ideas on what "molecular anthropology" is, with the help of an imaginary dialogue.

The use of comparisons of protein sequences for measuring evolutionary divergence in primates, to which most of this book is devoted, depends on acceptance of the concept of the "molecular evolutionary clock". This concept states that amino acid substitutions in protein molecules take place at an approximately uniform rate during evolution. There is much debate as to how "uniform" this rate is for a given protein. The general consensus is that it runs at different rates at various times.

Most molecular evolutionists use the "clock" to some extent because of its obvious relationship to phylogeny.

In chapter 2, Vogel, Kopun and Rathenberg discuss transitions and transversions in haemoglobin variants. They say that: "CT transitions in the DNA code corresponding to AG transitions in the mRNA code are more frequently observed than would be expected with random substitutions." So-called "CT transitions in the DNA code" are, however, actually base-pair substitutions in which there has been an interchange between an A.T pair and a G.C pair. The decision as to which DNA strand is transcribed depends on which strand contains the binding site for RNA polymerase; and this decision is unaffected by the amino acid composition of the protein that is subsequently synthesised. There is no way of deducing, from an amino acid substitution, which member of the corresponding DNA base-pair underwent the mutation, and which was changed when replication next took place. As the authors correctly note, their compilations are indeed biased, because haemoglobin variants are detected

by electrophoresis, which does not measure changes that are not accompanied by an alteration in charge. In their Table 9, Vogel *et al.* have written alanine codons as the complementary DNA codons. To be consistent with polarity of DNA strands, they should have been written from right to left. Also, one of the codons is wrong.

In Chapter 11, by Matsuda, Table 4 shows the amino acid exchanges and minimum mutation distances in the opposite order from the caption to the table. Chapter 17, by Goodman, presents a lucid account of primate genealogy as deduced from globins and cytochrome *c*.

The book contains extensive compilations of amino acid substitutions in homologous proteins, especially globins, obtained from various primates. It also contains chapters on random and non-random processes in molecular evolution, maximum parsimony, carbonic anhydrase, antibody specificity and gene action.

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## Expertise in analytical chemistry

*Analytical Chemistry: Essays in Memory of Anders Ringbom.* Edited by E. Wannien. Pp. xiv+607. (Pergamon, Oxford and New York, 1977.) £27.50.

THIS volume consists of a judicious mixture of authoritative reviews, stimulating and often entertaining essays, and a sprinkling of original papers. The topics are spread across the face of modern analytical chemistry with particular stress on those areas with which the late Professor Anders Ringbom has been most actively associated.

The most homogeneous section is the first, containing some sixteen articles on or related to the stability constants and structures of complexes in solution. They succeed in presenting a very adequate and well-blended picture of the present state of the art, with a significant proportion devoted to the question of mixed complexes which have been so actively investigated in the last decade. In the various reviews, the many technical problems which can so readily disturb or mislead the newcomer to this rather exacting field of study are laid out clearly and amply documented. This section should thus be of particular value to the non-specialist who wishes to update himself, but will I am sure also be read with considerable interest by those with long experience and greater expertise.

The second section is composed of eight articles on various titration procedures and three on colorimetric indicators. This is followed by four articles on aspects of electrochemistry (polarography, electrometric titrations and ion-selective electrodes), five on separation techniques (metal chelates in gas-liquid chromatography, ion-exchangers and extraction), seven on trace analysis (for example, activation, anodic-stripping voltammetry, fluorescence and atomic absorption) and three on kinetic methods of analysis. Finally, there is a miscellaneous section with articles on sampling, the writing of a scientific paper, photoelectron spectroscopy, statistical analysis, capillary electrophoresis, and the history of analytical chemistry.

The 600 closely packed pages provide a curious and varied mine of information and expertise in analytical chemistry, and the list of authors (for example, Kolthoff, Bates, Reilley, Freiser, Margerum, Elving, Laitinen, Anderegg, Beck, Nasanen, Osterberg, Perrin, Pribil, Bjerrum, Irving, Tanaka, Pungor, Almarin, Yatsimirskii, and Flaschka) contains many of the outstanding names in analytical chemistry. This book is a must for the appropriate libraries and should be placed in such a position that chemists, and analytical chemists in particular, are tempted to browse among its pages.

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● *Science, Technology and Society: A Cross-Disciplinary Perspective* (reviewed in the 10 November issue of *Nature*: 270, 126; 1977) is published for the ICSPS by Sage Publications: London and Beverly Hills, California.

● *Theory of Computer Science* (reviewed in the 3 November issue of *Nature*: 270, 85; 1977) is published by Chapman and Hall: London and distributed in the USA by Halsted: New York.

## Problems of desertification

*Desertification: Environmental Degradation in and around Arid Lands.* Edited by Michael H. Glantz. Pp. xiv+346. (Westview: Boulder, Colorado, 1977.) \$20.

THE United Nations Water Conference, held in Argentina in March, 1977, and the United Nations Conference to Combat Desertification, held in Kenya in August, 1977, reflect the worldwide attention now being paid to the destruction of arable or potentially arable land throughout the arid and semi-arid regions of the world. The volume under review comprises a collection of articles by specialists in a range of disciplines which examine and evaluate the social, political, economic, environmental and technical problems related to the causes and effects of desertification. Most of them are concerned, one way or another, with the Sahara and the Sahel savanna of Africa.

In the first of these articles, the editor outlines the approach of the United Nations to "desertification" or, as H. N. Le Houérou prefers to call it, "desertization". Glantz defines this as a global "environmental problem which is primarily national in cause and national in effect". The Sahelian states are among the poorest in the world and, therefore, "in need of financial, technical, moral and other support if they are to have any chance whatsoever to cope effectively with the problems that are linked to desertification within their borders". No-one, however, explains how one should refute the logic of the Sahelian peoples—if, during the last drought, two thirds of their cows died, then, by raising three times as many cattle, the original number might be expected to survive the next drought!

At least half of all the timber cut in the world is used as fuel for cooking or as a source of warmth. Eric P. Eckholm discusses the problem posed by an Indian official: "even if we somehow grow enough food for our people in the year 2,000, how in the world will they cook it?" The suicidal deforestation of Asia, Africa and Latin America will have to be reversed. The problem of ecological deterioration in Niger is discussed by James T. Thomson; that of pastoral development in Somalia by Jeremy Swift. Authors of other chapters include Richard W. Katz, William W. Kellogg, Stephen H. Schneider and Helen Ware. Surprisingly, the Interim Report of the South African Drought Investigation Commission of April, 1922, is reprinted,

without comment, as chapter 10. This historical document shows that neither today's problems, nor their solutions, are new—although the recommendation that jackals should be exterminated might no longer win universal approval.

The review of problems of desert land reclamation in the USSR, by A. G. Babayev, includes an appendix consisting of abstracts of 24 papers published after a symposium held at Ashkhabad, in 1976. Babayev concludes that scientists "must now concentrate on determining the optimum limits of harmonic development of society and nature". "The Communist Party and the Soviet government" seem to have learned that nature may be tamed, but cannot be conquered by man!

## Transport properties of simple liquids

*Classical Kinetic Theory of Fluids.* By P. Résibois and M. De Leener. Pp. xv+412. (Wiley: New York, London and Toronto, 1977.) £22.

"AMONG the many available texts on statistical mechanics, few, if any, give the reader a coherent and sustained introduction to the various methods that have made non-equilibrium statistical mechanics so successful." This quotation from the preface understates the difficulties facing a research worker in the field. If he tries to piece together the original literature, he meets mysterious concepts like "plateau time", "friction constant" and "auto-correlation functions"; and he can have a hard time in deciding whether a theory contains, or does not contain, adjustable constants.

This book contains a connected account of the various approaches to the problem of predicting the transport coefficients of a "simple" liquid. Nearly all the work is concerned with the rigid sphere and  $n$ 'th power repulsion models. Theoretical predictions are compared with "computer experiments" rather than with measurements on the rare gases. This is because of the complicated actual forces between real molecules of "simple" liquids, and also because information such as the time variation of correlation functions can be obtained directly from computer experiments, but can only be inferred indirectly from neutron scattering experiments on real liquids.

The only proper way of dealing with the famous "irreversibility paradox" is to proceed via Liouville's equation. This is done in section C of the book,

A book on deserts seems an unlikely place in which to find a discussion of the world's oceans, but J. Dana Thompson's interesting chapter examines the notion of biological deserts in the ocean, and assesses the limitations of the sea as a biological resource which, however, could be made somewhat more productive by mariculture, the reclamation of polluted areas, harvesting unconventional species, and the abolition of over-fishing. As in the case of all the problems of environmental degradation, we know *what* should be done but not *how* it could be accomplished. The matter is one of individual responsibilities.

**J. L. Cloudsley-Thompson**

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after two introductory sections on stochastic processes and on various forms of the Boltzmann equation. The Boltzmann equation has essentially irreversible solutions, and the central problem is to show that such an equation can be consistently derived from Liouville's equation by proceeding to the thermodynamic limit in a large assembly.

This programme cannot be carried through rigorously for any physically realistic model, but section C makes it clear that most, if not all, of the difficulties of principle have been gradually overcome, and that the remaining difficulties are almost certainly technical. The reader is led through some far from simple work with great clarity, and the style is pleasant and informative. No attempt is made to disguise the difficulties still outstanding—for example, the long term behaviour of the correlation functions and the fact that some treatments give transport coefficients that vary non-analytically with density (or diverge). Paradoxically, such difficulties seem to be particularly severe for two-dimensional assemblies. A final section discusses the, more formal, theory of transport coefficients based on time-dependent correlation functions.

The appearance of the book is most timely. It is splendidly printed and produced and there is a good index and some clear figures. The bibliography is surprisingly short but probably adequate, consisting largely of books and review articles rather than original sources. The authors have certainly achieved the objectives set out in their preface.

**H. N. V. Temperley**

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**nature**

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## The vulnerable side of science

SCIENCE is about people, the ideas they have, how they go about testing these ideas and how they persuade other people to accept them. Science is therefore largely concerned with communication.

Hardly the sort of definition that would get into a dictionary, which is more likely to speak of science as knowledge obtained by observation and experiment, tested, systematised and brought under general principles. Organised knowledge doesn't at first sound a very personalised affair—it has too obvious associations with the certainties of the periodic table and suchlike, or the neatness of textbooks. Yet every thinking scientist knows that just as 'coal' means a neatly stacked cellar of the stuff to the householder, but something highly personal, both friend and enemy, to the miner at the coalface, so 'science' is not only a neatly stacked cellar but also a complex and messy affair, a very human business with all its successes and failures, argument and consensus. This side of science is relatively poorly understood by the non-scientist. And even scientists themselves sometimes forget it when constrained to write short scientific papers or deliver ten-minute talks in which there is only room for the reporting of success, not the detailing of blind alleys, tortuous discussions, uncomprehending referees.

Because science is so much dependent on free and easy communication amongst its practitioners, anyone who interrupts the channels of communication used by scientists is capable of doing immediate and serious damage not just to those closely involved, but also to the advancement of science. Neutralise a fertile thinker in informal contact with tens, even hundreds of other scientists, and no-one else will be able to fulfil anything like the same role. And neutralising doesn't involve anything as vulgar as demolishing a particle accelerator or breaking up a laboratory. It simply means making one person difficult to get hold of and denying that person the normal means of communicating with the outside world. There are plenty of ways, both subtle and unsubtle, of achieving this, and the demonology is not restricted to governments.

It is, thus, encouraging that 1977 has seen a growing awareness by scientists of the importance of human rights issues, and a gradual realisation that talk of freedom of expression and interchange of ideas in high-flown and little-read international documents need not necessarily be meaningless but could be a lifeline, however fragile, to a beleaguered scientist. The report early in the year by the Council for Science and Society on scholarly freedom and human rights must be reckoned a major milestone in providing scientists with the necessary theoretical support to their practical actions and now, with the Belgrade review of the 1975 Helsinki European security conference drawing to a close, it is reasonable to wonder whether there are any signs that scientists are gaining or losing in their quest for the right to express themselves and communicate with each other.

Obviously there is no measure of such a complicated thing as freedom for scientists to be part of a global community, but even so there are few grounds for believing that things are getting better. Nor is tampering with scientists, and therefore with science, the preserve of any one power bloc or group of nations; there is gloomy news from many parts of the world. And lest it be thought that Western countries can be smug and self-satisfied in these matters, it should also be said that commercial secrecy and national security are often the justification by which *bona fide* scientists are gradually worn down into conformists, afraid to speak out on matters where they have high technical competence.

On the other hand there are favourable signs. Within the next few months it is expected that ICSU's Committee for Safeguards of the Pursuit of Science will come forward with some new proposals, and it is hoped that these will encourage the establishment of some centre to which scientists may turn for assistance or to which malpractices may be reported.

1978 marks the thirtieth anniversary of the adoption by the United Nations of the Universal Declaration of Human Rights. It could well be the year in which the simple, yet very vulnerable needs of the scientist at last get a heavyweight defender. □

# Stalin's scientific deputy addresses dissident meeting

THE inclusion of a symposium on scientific research in the Venice Biennale on culture has been described as an "act of considerable courage". Since the general theme of the 1977 Biennale was "cultural dissidence" it was hardly surprising that the round table on scientific freedom should include many who have shown considerable physical and moral courage in the defence of academic liberty. Voronel, Turchin, Plyushch, Dediu, Zhores Medvedev, the Papiashvilis, the Chudovskii brothers—the list of speakers reads almost like a role of honour of the dissident movement in science. Moral courage of no less an order, however, was shown by Dr Arnost Kolman, who, to an audience of active campaigners for scientific liberty, delivered a paper that, under the unassuming title of "The Adventure of Cybernetics in the USSR" described his part in Stalin's campaign to destroy free scientific progress, and replace it by a special party-orientated science.

Dr Kolman, at 85, is a living link with Lenin and the early days of Soviet power. His personal philosophy still remains Marxism, which he sees as having become under Stalin "the obedient servant-girl of political power". His picture of his destruction of science during those years is a horrifying one, since, he explained, it was carried out not by cynical careerists, but by people acting in good faith, but incompetent in the field in question.

Thus he, himself, trained in maths and physics, campaigned during those years against the prevailing philosophy, in defence of relativity, quantum physics, and mathematical logic. In life sciences, however, and particularly in genetics, he was fully prepared to accept the party dogma which pronounced genetics a pseudo-science. He

described how the editorial board of the philosophical magazine, *Under the Banner of Marxism*, of which he was a member, were given a mere three months to familiarise themselves with the theory and problems of heredity—and then to take up the battle against "mendelov-morganism", championing the "innovatory ideas" of Lysenko.

Kolman's paper was not only absorbing—as the *mea culpa* of a notable figure has always been fascinating from the days of Augustine of Hippo onwards—it also set the tone for the whole symposium by stressing the danger inherent on state interference in science. Zhores Medvedev gave a most lucid account of Soviet science policy during the last 60 years, several papers dealt with more recent events in Czechoslovakia (Frantisek Janouch, L. Durovic, and O. Poupa) and in Romania (Mihai Dediu). Kolman's revelations showed, however, that such interference need not necessarily be effected by bureaucrats or party apparatchiki, but by persons who genuinely believe that in doing so they are serving their country and the cause of scientific progress.

Against this background the more theoretical papers took on a significance transcending the current situation in the USSR and Eastern Europe. John Ziman's masterly exposition of the ethical principles underlined the concept of scientific liberty. Giorgio Bert on "Medical Science as an Ideology", Mark Popovskii on "Controlled Science", and Valentin Turchin's enquiry into the correlation between scientific training and involvement in human rights, all raised fundamental problems of the moral responsibility of scientists everywhere.

Likewise, in the special session on the political misuse of psychiatry, Dr Sidney Bloch noted that the revelation

during the last few years of Soviet malpractices had caused many psychiatrists throughout the world to reassess their personal ethical standards.

A number of speakers, in particular Giacomo Morpurgo, stressed the need for rapid and accurate dissemination of information of scientists subject to restriction and deprivation of academic liberty. The work of Amnesty International did not go unnoticed; but it was stressed that Amnesty campaigns only for those actually in prison. Owing to the rapid advance of science, exclusion from professional activity for more than a few months may produce irreversible academic "death". Hence the intervention of the world scientific community at the initial stages of academic restriction is a matter of considerable importance. A prime example of what can be achieved was the worldwide outcry against the campaign to expel Sakharov from the Soviet Academy of Sciences in 1973. Reports of specific efforts were received from Jeremy Stone (Federation of American Scientists), Tania Mathon (Solidarity Committee of French Scientists) and Goran Borg (Swedish Committee for the Freedom of Science).

Repeatedly throughout the symposium it was stressed that, although the theme of this particular meeting was repression and dissent in Europe, the problem of scientific liberty is indivisible and transcends all national frontiers. It is an integral part of the world-wide problem of human rights: if the cases of scientists command particular attention, it is because in this post-atomic age, the outcome of state misuse of science and technology can threaten the future of the entire world. The ethical responsibilities of scientists to their profession and to their colleagues are accordingly proportionally great.

Vera Rich



Arnost Kolman: a living link with the early days of Soviet power

## Sakharov warns of subtle pressure

The Soviet physicist Andrei Sakharov sent a personal message to last week's Venice Biennale. The following is an extract from his message:

"Liberty of opinion and exchange of information are of great importance throughout the world. . . . The new climate of international relations, called detente, has led to even more complex and varied links and communications, hence not only have the possibilities of positive influences been increased, but also the danger of the diffusion of more negative characteristics. This has increased mutual responsibility. . . . I consider the initiative of the Biennale to be very important, dedicated as it is to the problem of dissidents and non-official

creativity in the socialist countries. I myself belong to this scientific sector in which the ideological pressure of the state is nowadays not expressed entirely explicitly. . . . The epoch of the ideological struggle against the theory of relativity, quantum mechanics and cybernetics—the most shameful page in Soviet science . . . fortunately belongs to the past. . . . (Now) perhaps the most important thing is the humiliating position of the intelligentsia in the whole country, which is shown particularly in the inadmissible misery of the two most wide-spread intellectual professions—doctors and teachers—and in the total subjection of the intelligentsia to bureaucratic party control".



## New defence science chief offers cash to UK universities

UNIVERSITY and polytechnic scientists should become more closely involved in helping to determine strategies for defence research, according to Professor Ron Mason, the newly-appointed chief scientist to the UK Ministry of Defence.

Even five years ago, such a statement would have generated hostility from university colleagues and students alike. Both were concerned—for different reasons—with intrusions into the “purity” of research. Yet when Professor Mason’s appointment was announced at a meeting last week at the University of Sussex, where he has been professor of chemistry since 1971, even a student representative present greeted it, he says, with applause.

With the continued squeeze on university funding, and a growing acceptance of department-sponsored research, Professor Mason claims that there now exists a “more relaxed view” in universities about accepting military funds than a few years ago.

As chief scientific adviser—considered by many to be the highest position that any British scientist can hold in government—one of his main tasks will be to act as an interface with the scientific community. And the new atmosphere should, he feels, make this easier to achieve.

“One of the first things I want to engage in is a discussion on the relative balance of intermural and extramural research, and personally I would like to see a substantial increase, so broadening the base of expertise and advice,” Professor Mason said this week.

He emphatically rejects the notion canvassed by the Campaign for Nuclear Disarmament and others that university scientists should refuse to carry out military research on principle. A scientist’s responsibility should be to produce first-rate research in pursuit of aims determined by the conventional political processes.

“The proviso, of course, is that all research carried out on Ministry of Defence contracts should ultimately be publishable in the normal way; as a university man, I would be worried if an academic institution accepted any restrictions on publication.” Professor Mason has himself in the past received funds from the Microbiological Research Establishment at Porton Down in support of certain aspects of his work on ion transportation across cell



Ron Mason: “a more relaxed view” on military research

membranes, and feels no qualms at having done so.

“Another reason for wanting closer contact with scientists in universities and polytechnics is to open up discussions about long-term trends in defence research. And since these involve a mix of the scientific and technological with economic and political factors, a broad interdisciplinary discussion of objectives is required for which the ministry research establishments are not equipped.”

Professor Mason is no stranger to the world of science and technology policy, having been a member of the Science Research Council from 1971 to 1975, and chairman of its science committee for three years. He has also been a member of the requirements board of the Department of Industry since 1973.

One area in need of investigation in his new job is, he says, the systematic under-recruitment of scientists and engineers by the Ministry of Defence that has taken place in recent years.

“Whether a reluctance to take up a career in military research is due to the unattractiveness of Civil Service salaries in general, or of this type of work in particular, is at present unclear. But it might be a good idea for me to go out to talk to people in universities to help find the reasons for this situation.”

Professor Mason admits to being slightly “bewildered and overwhelmed” by the scope of his new job, which he takes up on 1 March, 1978 on a three-year secondment from the university.

He is convinced that the key to its success, at least in developing links between the scientific community and the defence establishment, lies in opening up discussion about military research; but whether closer liaison is acceptable to either party remains to be seen.

David Dickson

## Soviet beams over Sweden?

Is the Soviet Union developing a charged-particle anti-satellite weapon? Reports earlier this year listed evidence that tests of such a weapon had been carried out at a research facility near the Soviet city of Semipalatinsk, and described the debate raging between the US Air Force, convinced that the tests were being carried out, and the more skeptical CIA. A Swedish scientist has now published some findings that will add to the speculation.

Dr Lars Erik de Geer of the National Defence Research Institute detected traces of radionuclides neptunium-239 and molybdenum-99 in the atmosphere over southern Sweden on five occasions during the first half of 1976. The same atmospheric samples also contained small amounts of fission products iodine-131 and barium-140. On the first two occasions—in late February, and March—the prevailing winds had been from the east during each period of three days when the nuclides had arrived. On the other three occasions—in April, May and June—the winds had blown from the east on at least two days in each week during which the nuclides came. Dr de Geer thus postulates that “the material arrived in Sweden by way of southern Finland or western USSR and the Baltic Sea”.

What could have caused such activity? The short answer is: no known source. The amounts of neptunium and molybdenum detected are compatible with the ratios found in debris that circulates for a few weeks after the explosion of a nuclear bomb. But, as Dr de Geer points out, if these nuclides resulted from some fission process, it is strange that other short-lived fission products (such as tellurium-132) were not also detected. Neither is the composition of the samples consistent with discharge from an ordinary nuclear power reactor. He wonders whether some laboratory experiment involving fresh fission products could have been responsible; but a check on research laboratories in Sweden, Denmark and Finland showed that no such work was in progress during the period in question. The





most exotic explanation—that the nuclides were produced by charged-particle beam experiments in the Soviet Union—seems to fit in that the observations were made at the same time as the experiments at Semipalatinsk were reportedly started. According to meteorological trajectories, however, nuclides from this area of the USSR could have been carried to southern Sweden in March, April and May, but probably not in February or July. This means that, although the nuclides detected in February were deposited by an easterly wind, they probably came from a source other than Semipalatinsk. Given the nuclides' short half-lives (ranging from 2.35 days for neptunium-239 to 12.79 days for barium-140), and the distance from Semipalatinsk to Sweden (about 4,000 km), the winds carrying the nuclides would have to have been strong. Although meteorological data suggests that the winds probably would have been strong enough, sufficient doubt remains for Dr de Geer to cite this as a factor against the Soviet source.

Dr Kay Edvarson, of the National Institute for Radiation Protection, comments that it is impossible to pinpoint a unique source for such activity. "We have fairly extensive surveys made now on releases from power reactors, and Dr de Geer's findings don't really fit any reactor types known to us. The National Defence Research Institute quite often detects some radioactivity which can not have come from a nuclear test. These samples are generally assumed to have come from small, localised sources such as a hospital. But it is impossible that the sort of activity Dr de Geer detected can have come from such a source. On three occasions the nuclides were detected at several sampling stations in southern Sweden, so it's highly improbable that the source was localised. It has to be reasonably far away, and strong. If it had been a strong source somewhere in the Nordic countries or West Germany, we would have heard about it." He points out that the probability of detecting the sorts of samples now under discussion is small.

"They would be masked by fairly fresh debris from a nuclear test", he says, "and the release time has to coincide with the wind pattern".

Dr Bhupendra Jasani, of the Stockholm International Peace Research Institute (SIPRI), says it is difficult to think of any sources other than those mentioned by Dr de Geer which might have caused the activity. "I am very skeptical of those who claim that the Russians have a charged-particle beam weapon in operation", he says. "However, the Soviets are pioneers in accelerator physics and accelerators, and they could well be working on such weapons—just as the Americans may be." Dr de Geer's report, published in *Science*, quickly drew a statement from the Pentagon questioning the hypothesis of the Soviet source. But, as Dr de Geer points out, "I am not saying that the nuclides came from charged-particle beam weapon tests in the USSR. It is simply that, as no other source seems to fit, the possibility that they did has to be considered."

Wendy Barnaby

## Sweden follows US example on aerosols

SWEDEN is to become the first country in Europe to ban the manufacture and use of chlorofluorocarbon (CFC) propellant gases in aerosol sprays.

The ban, which will take effect in 1979, reflects public concern at the possibility that such gases may deplete the stratospheric ozone layer, hence potentially reducing the atmosphere's ability to screen ultra-violet rays, and leading to an increase in skin cancer.

In the US, fears that the extensive use of CFC gases could cause a decrease of ozone of up to 14% over a period of 100 years have already led to a similar ban, the result of a joint decision by the Food and Drug Association, the Environmental Protection Agency, and the Consumer Product Safety Committee.

Sweden is the first country in Europe to follow the American example. The Swedish Government's action is based on a recommendation of its Products Control Board that a licensing system should be introduced for aerosols, and that such licences be withheld from most aerosols using CFC gases.

The manufacture and import of such sprays—excluding certain products required for special uses such as medicine—will be banned from the beginning of January 1979, and their sale from the following June.

The move has already brought reaction from a number of Sweden's trading partners in the European Free Trade Association (EFTA), concerned at the economic impact it may have. Finland,

for example, which exports many aerosols to Sweden, has asked Stockholm either to withdraw the ban, or to extend the timescale considerably.

The Finnish request is based on the position held by the majority of Euro-



"It's an aerosol for getting rid of Finnish aerosol salesmen"

pean countries that although CFC aerosols represent a theoretical danger to the ozone layer, no substantial evidence has yet been produced to indicate that any significant depletion of ozone does indeed take place.

Widespread public controversy has surrounded the issue since the possibility of such depletion was raised by

Rowland and Molina in 1974. It is this concern that has led to the current ban in the US, where many manufacturers are turning to the use of hydrocarbon gases as an alternative.

No theoretical model of the chemical interaction between ozone and CFCs in the atmosphere, however, has yet produced an adequate representation of what actually happens. In particular, most models rely on a one-dimensional interaction, and are hence unable to take into account the full three-dimensional movement in the stratosphere.

In view of the scientific uncertainties, countries such as Britain, Germany and France have decided that more research is needed before a decision about whether or not to ban CFC aerosols. The EEC has recommended that a review of the situation be made in the second half of 1978, and has also commissioned the UK-based consultants METRA to carry out an economic impact study on the effects of a ban.

The Swedish government has therefore decided to break ranks with other European countries, and in particular its Scandinavian neighbours. However the decision is likely to be welcomed in the US, where FDA commissioner Donald Kennedy said earlier this year that since the threat of ozone depletion was a global problem, he hoped other countries would follow suit.

An official of the Swedish Department of Industry said in Stockholm last week. "The Swedish philosophy is that this will take a long time, and someone has to take the lead."

David Dickson

## Bangladesh reviews science policy

MODERN science and technology was introduced to the Indian sub-continent by the British during the late 19th century. It developed in only a few areas of present day India, no significant development taking place in those areas which now make up Bangladesh and Pakistan. A national policy for science and technology development there was initiated as late as the 1960s. Planning and executive agencies were subsequently formed and some institutions for scientific and technological education and for research and development were established. Over the last ten to fifteen years an indigenous scientific and technological infra-structure has begun to be built up in Bangladesh.

The need for an integrated science and technology policy in Bangladesh has led to the formation of the National Council for Science and Technology (NCST) to advise the government on formulating policy, and planning, coordinating and evaluating science and technology development. In concrete terms the NCST determines national objectives, formulates strategy and identifies areas where science and technology can contribute to national development. The talks also include coordinating and evaluating scientific and technological programmes in various sectors, and the development and motivation of scientific and technical manpower.

The government of Bangladesh has now undertaken economic development programmes to raise the standard of living of the people and improve socio-economic conditions. Implementation of these programmes needs specific technological capabilities. Any assessment of future possibilities and formulation of appropriate development strategies must be based on an analysis of national strength and weaknesses which should take into account the potential contribution that science and technology can offer. Science and technology policy for both short and long term strategies, thus, constitutes an integral part of the country's overall development plan.

With a view to planning and programming scientific and technological developments effectively, the NCST has undertaken to establish a data base on manpower, and the funding, management and facilities for the existing scientific and technological infrastructure. This would involve surveying existing scientific and technological personnel and stock taking of the research institutes, laboratories, workshops and supporting facilities. The current programmes in these institu-

tions are to be reviewed and plans for the development of existing scientific and technological institutions are to be made on the basis of the results of these surveys and reviews. Further, NCST intends to undertake studies and make recommendations on the organisation and modes of financing of the research councils, institutes and other supporting facilities.

On the development of human resources in science and technology in Bangladesh, NCST proposes to review the existing education and training programme and suggests appropriate measures for their expansion and improvement. Recruitment and promotion rules for scientific and technical personnel with appropriate measures for proper incentive and motivation. The government has already instituted a fellowship programme for science, engineering, medical and agricultural graduates who cannot find suitable employment inside the country or experts working abroad but interested

in joining the institutions of Bangladesh.

NCST intends to undertake a comprehensive scheme for preparation and production of science and technology text books in Bengali and reference books in both Bengali and English up to the highest level of education and to advise the government on its phased implementation. Popularising science and technology and disseminating scientific knowledge among the public, particularly on the human implications and social impacts of science and technology will be one aim. In the long term these studies should lead the government and other concerned agencies to formulate the philosophy, guidelines and workplan for a course of action.

Bangladeshi science and technology policy also envisages longterm studies on the development of scientific and technological potential, appropriate or alternate technologies, policy guidelines for international and regional cooperation and promotion of associations, academic and professional bodies and human rights for scientists.

**M. Kabir**

## FAO conference embraces third world

WHEN, in January 1976, Edouard Saouma, a Lebanese Christian, took over as Director General of the Food and Agriculture Organisation (FAO), there was a good deal of headshaking among the old hands in international agriculture, and when he announced his policy for improving the efficacy of the organisation, there was even more heart-searching among the senior staff. His policy was aimed specifically at more direct contact with ministries of agriculture and development in the Third World countries.

The FAO biennial Conference, which ended early in December at the headquarters in Rome, must have given Saouma a good deal of satisfaction. After two years of his regime, government after government expressed approval and encouragement for the policy at which many of their representatives had looked askance early in 1976. Nor did this approval come only from the Third World countries towards whom the new policy has been especially directed.

The most far-reaching of Saouma's ideas has been the Technical Cooperation Programme. Seen initially by many as a duplication of effort with the UN Development Programme (UNDP) this has now proved its ability to provide small-scale assistance for specific projects at very short notice—something the cumbersome machinery of UNDP has never been able to provide. In its first year, 190 projects have been approved under this programme. The success of

this scheme certainly owes a good deal to another innovation, much criticised when announced: the appointment of FAO representatives to certain countries, again in order to provide direct and rapid contact in critical areas.

Two other activities came in for special approval from many delegates in Rome. One was the Seed Improvement and Development Programme, fundamental to increased production in the developing countries. The other is the campaign against post-harvest food losses—something for which every FAO Conference has asked since the organisation came into being. Since it is estimated that cutting such losses by half could save up to \$7,500 million a year in the foreign exchange for the developing countries, the amount allotted for initiating this campaign, a mere \$10,000, seems modest indeed. It is hoped to double this with external contributions.

Referring to the 1979 World Conference on Agrarian Reform and Rural Development, Saouma said that he wishes to "avoid confrontation, rhetoric and confusion"; many observers must have noted that he certainly managed this at his own Conference, at a time when politicising and backbiting have come to be a normal part of such major international meetings. This is perhaps another good augury for his ability to control the spending of the \$237,377,000 he is asking for his regular budget for the biennium 1978-79.

**Peter Collins**

## Australian election leaves uranium issue confused

**L**AST weeks' general election in Australia scarcely changed the massive majority held by the Liberal and National Country Party coalition government. Nor has it changed the odds on whether or when Australia is to become a major exporter of uranium.

At first sight this observation may seem paradoxical—even perverse. Surely such a total victory must have fortified Mr Fraser and his government in their confrontation with the opponents of uranium mining? This certainly seemed to be the view of the uranium mining companies expressed through their lobby organisation, the Australian Uranium Producer's Forum. Within hours of the election result being known the forum's chairman declared that the Fraser government now had a mandate for its uranium export policy. Unfortunately even if one accepts the dubious mandate the mining companies' view is without foundation.

While the government parties polled about 48% of the national vote, the combined vote of the two main parties advocating a moratorium on uranium mining, the Australian Labor Party and the Australian Democrats, was almost 50%. Furthermore, it would be hard to argue that the uranium issue was at the forefront of most voters' minds as they went to the polls. Although it had been seen, before the event, as a crucial issue in a December general election, in the event uranium received relatively little attention during the campaign. The government parties, in particular, seemed to go almost out of their way to avoid discussing uranium and nuclear power. In an opinion poll conducted eight days before the election, one of the more respected polling organisations found that only 5% of voters considered uranium export to be the most important election issue. Thus the election result is in no sense a positive endorsement by the majority of Australians of the government's policy on uranium.

### Government undeterred

This fact is unlikely to deter the government from attempting to claim a mandate for its policy. And the election result is certain to increase its confidence in seeking a confrontation with those who oppose it, which means, principally, individual unions or the trade union movement as a whole. There has been considerable speculation about when and how such a confrontation may occur. In the transport

of yellowcake, unions have already taken industrial action on uranium.

The most crucial of the unions are almost certainly those in the building and engineering industries, whose members will have to be involved in constructing the mine and mill. These unions are mostly large, well organised, and strongly opposed to the mining and export of uranium. They seem certain to be in the vanguard of any confrontation with the government. While it is obvious that a great many factors will determine the outcome of a confrontation, should it occur, the support which the unions receive from the labour movement as a whole and from the general community will clearly be of great political importance.

There is now a large and articulate opposition to uranium export, co-ordinated nationally through the movement against uranium mining. While not yet as large as the movement opposing the Vietnam war was at its peak, the opposition to uranium mining appears to be both better organised and politically more sophisticated.

Up till now Mr Fraser has treated the uranium issue with great circumspection. His failure to use it as an election issue could be taken to indicate uncertainty about the popularity of his policy. In explaining the policy, he emphasised that following the reports of the Ranger Uranium Environmental Inquiry (otherwise known as the Fox reports) he has demonstrated in both word and action the importance he attached to improving safeguards against nuclear weapons proliferation. All these moves may have been effective in reassuring uncommitted opinion on the uranium issue; though if Mr Fraser was hoping that he would also placate his opponents, he must have been disappointed.

However, there are now signs that the government is being less cautious in pursuing its uranium export objectives. For example, it has so far failed to provide the necessary finance and administrative support for the team appointed to carry out environmental research and supervision in the Alligator Rivers region of the Northern Territory, where the major uranium deposits are located. While the hold-up may simply be the result of bureaucratic inertia or parliamentary delay, the opponents of uranium mining are inclined to see it as presaging the breaking of promises on environmental protection.

Much more important, at least in the international context, was the state-

ment in the last week of the election campaign by the Deputy Prime Minister and Minister for National Resources, responsible for uranium in Mr Fraser's pre-election government, Mr Anthony. He announced that the government was reconsidering its policy of prohibiting the reprocessing of spent reactor fuel made from Australian uranium, and does not wish to rule out reprocessing by 'acceptable' countries. This change in policy would make it difficult for the government to continue to argue that by exporting uranium it was furthering President Carter's policy of preventing reprocessing and the plutonium economy by providing ready access to supplies of nuclear fuel.

Mr Anthony has consistently been far more blunt than Mr Fraser in his support for uranium mining and his statement may not have the support of Mr Fraser. In the immediate aftermath of the election it is not certain that Mr Anthony will retain responsibility for uranium development in Mr Fraser's new government. However, his statement undoubtedly reflects the problem the government is having with its policy on proliferation. President Carter's policy on nuclear fuel reprocessing is clearly in some difficulty, and may be abandoned. More important from the Australian government's point of view will be the firm commitment to reprocessing by its major potential uranium customers: Japan, West Germany, and the UK.

### Economic question

Finally, there is the question of the economics of uranium mining. Canadian officials involved in making decisions on the development of the new uranium prospects in Saskatchewan recently expressed amazement at what they considered to be highly optimistic expectations of both sales volume and price of uranium held by the Australian government and mining companies. The uranium debate in Australia does not so far appear to have comprehended the considerable down-turn in uranium market prospects over the next ten years or so, which has taken place recently. When the Australian people realise that an immediate start to uranium mining may not bring the great economic benefits they have been led to expect, they may be less willing to support the government in any confrontation that may occur. It is noteworthy that one of the few seats to record a significant swing against the government was Northern Territory, where much publicity has been given to the possible economic benefits of uranium mining. There is still a very long way to go before Australia becomes a major uranium producer.

**Hugh Sadler**



# correspondence

## Letter from Argentina

SIR,—I welcome the concern Louise Harel, Jose Uriel and Jean-Claude Salomon show to my countrymen (3 November, page 8) by inviting professionals not to participate in the twelfth International Congress of Cancerology to be held in Buenos Aires, in October 1978.

However, while I believe that sincere international solidarity can be of great help in the fight for universal human rights, and while I welcome the writers' desire to see democracy upheld, I believe their proposal in this case is misguided.

Many countries are suffering from terrorism. In Argentina scientists, professional people, students, workers, policemen and the military, priests, women and children have been affected. It is true that there is a long list of people who have disappeared or been kidnapped and that some have been forced to emigrate by intimidating threats and torture. But action is also being taken against all kinds of terrorism. President Videla has repeatedly declared that the state is the only force charged with the security of people and that any illegal repression will be severely punished. He has also expressed the need for exchange of opinions if Argentina is to be based on a renewed, stable and progressive democracy.

These concepts are shared by the Permanent Assembly for Human Rights and by the Argentine League for the Rights of Man, both functioning legally. Political personalities, cultural and scientific representatives, as well as others from all branches of activities, publicly condemn the terrorist violence and call for an end to persecution and arbitrary repression.

But is a decision to boycott the International Congress an appropriate way of expressing solidarity? Is it in effective way of helping us reach a democratic goal? I think not. One of the objectives of terrorism is to disorganise the life of a country, to create a climate of intimidation and to isolate the nation from the international community. A boycott of the Conference would contribute to this isolation and would favour the plans of terrorists to promote chaos. The fight against terrorism should stem from the organised way of life of the country itself, which will break up antisocial manifestations. The government has assumed this

responsibility by proclaiming its monopoly of repressive actions against terrorism. It is also the responsibility of the democratic forces which speak out for the right to work in peace and liberty. This is what scientists, professional people, workers and their institutions and associations are doing. All these activities are proceeding in spite of economic restrictions and social violence and they should be supported.

No matter how great the concern of our colleagues abroad, Argentinians are far more concerned about showing support for security and peace. It is precisely to show solidarity with our efforts that foreign scientists should come to our country, should acquaint themselves with our complex reality, should make their contributions towards the fight against cancer and should acquaint themselves with the Argentinian work on the subject.

I am making this appeal as a scientist who has been dismissed from his position in the National Council for Scientific and Technical Research (CONICET), for "service reasons", without any justification. I have not emigrated, neither have most of those affected. I have asked for reconsideration and I will continue my fight for justice. I am going to participate in the International Congress of Cancerology with my modest contribution under such difficult circumstances. I am confident that the patriotic forces of our country, both military and civil, will find a way of effectively restoring democracy. All honest Argentinians are striving for this end. The presence of foreign scientists at the Congress will be an expression of respect and solidarity with our human and scientific endeavour when we need it most.

EMANUEL LEVIN

Buenos Aires, Argentina

## Go to Argentina

SIR,—Once more the European scientific community is threatening to boycott the scientists of a specific country because of the type of government they have ("Petition for Argentinian scientists", 3 November, page 8). I think this is unfair to the great majority of scientists and students of the particular country. I would like to comment on this problem by taking the example of Chile, my native country.

As a small country with a low population, mainly supported economically by copper exportation, Chile used

to have the reputation of being an example of democracy in a continent where, together with two or three other countries, it was the exception. Its universities had excellent research groups in many disciplines able to compete with scientists from much richer countries.

All this was jeopardised by the 1973 military "putsch"; the research effort was severely affected but not killed (see "Science in Chile since Allende", *Nature* **265**, 486, 1977). Many Chilean scientists left the country, some because of direct persecution, others because of political ideas or moral convictions. The first of these groups was composed of scientists who had key positions during the 1970-73 period, or who were considered to be important by the "new lords". Many of these people were imprisoned and then went abroad through embassies. In the second group are many scientists who had already been abroad and for whom the possibilities of obtaining good positions in the USA and Europe were favourable.

Some university departments had to close down because all the teaching staff had left. This was the case for disciplines like physics and mathematics. In the field of biochemistry, however, an important number stayed, although many left. Who are these women and men who did not leave?

Are they all fascists as is generally assumed, mainly in Europe? One thing is clear to me: the longer they remain isolated from the rest of the world, the more likely it is that they will become convinced by the government of the so-called "general Marxist confabulation against Chile". There may be a small fraction of native academics that is sympathetic to and even enthusiastic about the "junta", but most of the Chilean scientific community is made up of convinced democrats who have supported leftist or left of centre political positions. For professional or familial reasons they have stayed in Chile.

What happens when an invitation or the opportunity to visit Chile is open to a foreign scientist? Immediately thousands of voices full of fury are raised to protest against such support of the authoritarian regime. This is extremely evident among European unions or intellectuals. Of course, other annual exchange programmes organised by certain European governments with other dictatorship-ruled countries do not raise such a reaction.

The attempt to isolate Chile reminds me of a similar attempt to isolate Brazil a few years ago. Although the Brazilian government is still very authoritarian, scandals are no longer caused when foreign scientists are invited to Brazil. Moreover, the great majority of the Brazilian scientists are opposed to the military government (although many of them returned to Brazil after the military took over, because of very important salary rises).

The main victims of this isolation, at least in the case of Chile, were the common people and more specifically scientists and students. The Chilean government, less stupid than generally thought, has used the argument of "all against us" as a powerful nationalistic tool. It seems aberrant to me that while scientists from all over the world are being persuaded from going to Chile, a superpower, which originated and supported the isolation campaign, is engaged in an exchange of political prisoners with the Chilean government, giving a tremendous popular credit to the military junta inside the country. A visit of all Nobel laureates to Chile, would not strengthen the military as the exchange of prisoners did.

Attending an international scientific meeting or visiting a Chilean or Argentinian university is not synonymous with supporting the military government of these countries. On the contrary it is a unique opportunity to contact scientists and students and to encourage and help them to overcome the scientific and moral isolation they suffer.

We have to remember the case of Spain, a country which until a few years ago had the same type of government as Chile and Brazil now have. As long as it was isolated from the world no hope of ending the dictatorship existed. As more and more foreigners entered the country, an irreversible flow towards democracy started.

No tourist boom can be expected in Chile or Argentina, but scientists and academics passing through can be an indispensable link with students and scientists. Without this bond, they may be convinced by the chauvinistic arguments used by the government each time a world campaign is organised against it.

This is a plea not to support the South American dictatorships or to ease the criticisms against them but to maintain a link between their scientific communities and the rest of the world. This may be the unique possibility of keeping alive the result of years of effort, and the only hope of evolution towards the revival of democracy in these countries.

SIMON LITVAK

University of Bordeaux France



**Competition 15** asked for an appropriate quote for our front cover. A good varied entry with an honourable mention to D. Irwin (Boston) for "Pereant qui ante nos nostra dixerunt" (May those perish who have said our things before us). But £10 goes to the Mammalian Development Unit, University College, London

## Technik or technics

SIR,—On most topics, I would not set out to disagree with those who write to support me. However the question is too important to let go: it concerns the separateness of the German-language notion of a third culture of *Technik*.

Rey and I (1 September, page 2) argued that the idea of *Technik* represents a missing concept in English, whereas Cavalier-Smith (20 October, page 646) would like us to follow Lewis Mumford instead and revive the English word "technics". We avoided "technics" because Mumford got things very wrong in the book referred to, *Technics and Civilisation*.

Notably he argued for a distinction between the "neotechnics" of the age since about 1880 or so, and the "paleotechnics" which went before. Of the period just before neotechnics, he wrote that its inventions "came into existence, for the most part, without its [science's] direct aid"; whereas later in the "neotechnic phase, the main initiative comes, not from the ingenious inventor, but from the scientist who establishes the general law: the invention is a derivative product".

Detailed investigation does not support this viewpoint, or the distinction between ages of manufacture on which Mumford's thesis is founded; science has always influenced manufacture, but never controlled it. Neither does detailed investigation support the viewpoint of R. J. Forbes, the author of *Man the Maker* (1950, Shuman), another hero of the historians of "technology". He writes his subject up, most misleadingly, as an account of the exploits of "applied science" through the "conquest of nature". A reliable book of the influence of *Technik* on the culture has yet to be written.

I am absolutely at one with Cavalier-Smith, however, that there is only harm to be done to science in the long term, through a blurring of the distinction between science and the technical

who communally turned up: "Is this," I cried, "the end of prayer and preaching? Then down with pulpit, down with priest, and give us Nature's teaching!" (Whittier)

**Competition 16.** To the uninitiated, many scientific pictures are ambiguous: a section through a cell could equally be a slice of a lunar rock. Readers are invited to give a misleading caption to any picture that has appeared in, or on the cover of, a recent *Nature*. £10 to the winner. Entries to Competition 16, *Nature*, 4 Little Essex Street, London WC2 by 1 February 1978.

functions of manufacture. One vehicle for this harm has been the ghastly construction called "technology"; the only "-ology" in history to make useful, bulky artifacts as a primary output, a word which outsiders to manufacturing use when they want to hide their ignorance of it.

M. FORES

London, UK

## Sunflower's siesta

SIR,—Observations on the sunflower's night life (8 September, page 102) prompt me to put on record a piece of its daylight behaviour which I have never seen described before. In Turkey, in July this year, I consistently saw that at 1 pm on bright days the flowers all faced the sun but by 2.30 pm all heads had turned away and faced north. At about 5 pm they again began to face the evening sun and ended the day facing west.

Perhaps even the sunflower finds the Turkish afternoon summer sun a bit hot and has a thermal switch overriding the sun-tracking mechanism, so that it can have a siesta. Is the siesta phenomenon unusual, or do sunflowers always behave like this in hotter climates?

ROBERT G. MILNE

Torino, Italy





# news and views

## Immune surveillance revisited

from R. W. Baldwin

THE immune surveillance theory as developed by Burnet (*Immunological Surveillance*, Pergamon Press, Oxford, 1970) proposes that a central role of the immune response is to provide a natural defence against cancer and this was later developed to emphasise the importance of thymus-dependent lymphocytes. It was, therefore, more than a little disconcerting to find that congenitally athymic (nude) mice do not have an increased incidence of spontaneous cancers and they are no more susceptible than conventional mice to chemical carcinogens (reviewed by Stutman, *Adv. Cancer Res.* **22**, 261; 1975).

It is axiomatic, however, that nothing in tumour immunology is simple and it has recently been proposed that whereas T lymphocytes may be an important component of the responses induced by immunological manipulations, such as immunotherapy, they may not have a central role in more natural conditions such as those involved in recognising clones of transformed cells. Instead, attention is being focused on so-called 'natural killer cells' since these fit almost exactly the immunologists' design for a surveillance cell in being able to recognise and kill malignant cells without previous sensitisation. The basic observation which drew attention to natural cell-mediated immunity arose out of quite extensive studies comparing the *in vitro* cytotoxicity of peripheral blood lymphocytes from cancer patients and normal donors for cultured cancer cells. Initially these investigations were interpreted to show clear-cut specific cytotoxicity by 'sensitised' lymphocytes from patients, but in later studies by Takasugi *et al.* (*Cancer Res.* **33**, 2898; 1973) and Oldham *et al.* (*J. natn. Cancer Inst.* **55**, 1305; 1975) it became apparent that lymphocytes from normal individuals, who would not be expected to have been exposed to the relevant cancer-associated antigens, were also cytotoxic. Moreover in many

instances the natural cytotoxicity of lymphocytes from normal individuals was even greater than that seen with similar preparations from cancer patients. Natural cell-mediated immunity has since been examined using more closely controlled animal systems and, as in humans, lymphocytes from normal mice and rats have proved to be cytotoxic *in vitro* for tumour cells, especially those of lymphoid origin.

In the mouse and rat, normal killer (NK) cells have been detected in most lymphoid organs, with particularly high activity in the spleen, lymph node and peripheral blood whereas in humans, studies have mostly been restricted to peripheral blood lymphocytes. In mice NK cells develop in the absence of the thymus and appear and disappear in a highly typical manner, reaching peak levels at between 5 and 8 weeks of age. NK cell activity is also under genetic control, allowing the classification of low and high NK cell strains. The position in other species, especially humans, is still unclear, but it has been proposed that NK cell activity may be related to HLA phenotype. The characterisation of NK cells is more controversial and it is this aspect of the problem which leads to most confusion. In the mouse, the NK cell is thought not to be a mature T cell because of its presence in athymic mice, although Herberman and Holden (*Adv. Cancer Res.* **27**, in the press) have suggested that it might be a primitive T cell. The characteristics of NK cells in humans are even less well understood and at present the only point of general agreement is that these cells are not macrophages.

Although it has been implied that natural killer cells may provide the host with a natural barrier against malignant cells, evidence on this point is sparse. The resistance to growth of transplanted tumours, including those of human origin, in athymic nude mice has been correlated with their high levels of natural cell mediated cytotoxicity. This evidence is not sufficiently compelling, however, since

these animals have been reported by Pimm and Baldwin (*Nature* **254**, 77; 1975) to be able to reject tumours through macrophage-mediated reactions. More convincing are studies by Kiessling and his associates (*Int. J. Cancer* **15**, 933; 1975) correlating the growth potential of transplanted tumour cells in different strains of mice with their levels of NK cell activity. Also within one strain Sando *et al.* (*J. natn. Cancer Inst.* **55**, 603; 1975) found that resistance to a transplanted tumour changed with age in a pattern similar to the known age-dependent variation of NK cell activity. These approaches have been further extended in a study now published by Haller *et al.* (this issue of *Nature*, page 609) which provides more direct evidence for a role of NK cells in suppressing tumour growth. Mice were thymectomised, irradiated and reconstituted with anti-T-cell treated bone marrow cells or foetal liver cells. When lymphoid cell donors of a compatible substrain of high NK cell activity were used, the transplanted tumours were rejected, whereas no resistance was observed in mice receiving cells from donors with low NK cell activity. These findings point to the potential of NK cells in tumour resistance, although whether they have a 'decisive role' remains to be established. In this context, it is pertinent that cells with similar characteristics to NK cells can be isolated from tumour biopsies, and that bacterial vaccines such as bacillus Calmette Guérin (BCG) and *C. parvum* which are being widely used in cancer immunotherapy trials markedly influence NK cell activity. One should guard against a too unitarian view, however, since many of the approaches for enhancing nonspecific immunity against cancer by the use of bacterial vaccines are known to require macrophages. Nevertheless the concept of a nonspecific lymphocyte which recognises aberrant cells is attractive for the immunosurveillance concept and augmentation of these cells could have therapeutic potential. □



# Are comets dirty snowballs or dust swarms?

from David W. Hughes

HEATED debates in science are always fascinating and none more so than the long standing one between cometary scientists as to whether comets are made up of a swarm of small, meteoric dust particles or a single kilometric sized icy nucleus. More fuel has been heaped on the fire recently by Lyttleton (*Q. Jl R. Astron. Soc.* **18**, 213; 1977) and Fellgett (*The Observatory* **97**, 23; 1977).

Both authors base their argument on that well worn principle of scientific methodology first propounded by William of Occam in the fourteenth century. Occam's Razor intimates that theories should be based on as few hypotheses as possible. Hypotheses must not be invented merely to explain away difficulties, especially if those hypotheses are not suggested by other features of the phenomena under investigation and cannot be tested in any way.

## Swarm or macronucleus?

How does this apply to comets and especially to the debate between the swarm enthusiasts, of which Lyttleton and Fellgett are the most enthusiastic, and the large army of macronucleus supporters? First, I shall explain the two concepts in more detail. The classical view, held by most people up to the early 1950s, is that a comet is a vast swarm of tiny particles separated by very large distances—around  $10^{25}$  particles of mean size about  $10^{-2}$  cm separated by tens of metres. The average comet diameter is about 100,000 km, average mass around  $10^{18}$  g, spatial density less than  $10^{-12}$  g cm $^{-3}$ . The icy conglomerate model was formalised by F. Whipple in the early 1950s (*Nature* **263**, 15; 1976) and since then it has gained pre-eminence. Here the fount of cometary phenomena is a single kilometric sized body containing about 25% by mass dust particles, the rest being ices of methane, ammonia and carbon dioxide embedded in H $_2$ O snow as hydrates or clathrates. A comet of mass  $10^{18}$  g would have a nucleus about 6 km across with a density around 1.1 g cm $^{-3}$ .

Both these models are hypothetical and as such Occam's Razor seems powerless to distinguish between them. The swarm is so tenuous that it would

have to extend over  $10^7$  km in the line of sight before it ceased to be transparent. A solid nucleus of length 1 km transverse to the line of sight at 1 AU would subtend an angle of less than 0.002 arcs at an Earthbound observer and is thus way below the resolution of all present day telescopes. So both are unobservable.

## Cometary origin

Hypotheses on cometary origin also seem powerless to distinguish between the two models. Swarm comets are supposedly formed by accretion from an interstellar cloud. Particles streaming past the Sun become gravitationally focused downwind, lose energy by inelastic collisions, are retarded to velocities below the escape velocity and then become gravitationally attracted to the Sun, slight perturbations preventing them actually falling into it. Dirty snowball comets are supposedly the remnants of an enormous family of planetismals, some of which coalesced to form the planets Uranus and Neptune. These cometary planetismals were perturbed (by planetary encounters) out of their original orbits to the Oort sphere, a great (but again unobservable!) comet reservoir some 40,000 AU from the Sun, from which they are periodically perturbed by passing stars into orbits with perihelion passages among the planets. Both theories are possible but obviously it requires only a small addition to the interstellar accretion theory to produce icy nuclei anyway. The fact that diffuse interstellar dust and gas clouds are considered to be the parents of stars and planets (and we should at least be convinced that planets exist) does not put this beyond the bounds of possibility. If the planetesimal theory is true, all comets are as old, if not slightly older than the planets (giving them an age just over  $4.6 \times 10^9$  years) and the number of comets in the Solar System must have been decreasing ever since. Fortunately icy nuclei comets can be kept in 'cold storage' in the Oort sphere. Accretion mechanisms have the advantage that the comet family can be enhanced every time the Sun passes through a dust cloud. Here comets can have a wide range of ages.

It is to be hoped that the observations of cometary phenomena can distinguish between the two models. Evidence is needed that points unequivocally at one or the other (or neither).

Unfortunately both models can explain many phenomena with reasonable success. The coma can be formed by desorption from the solid meteoroids in the swarm or by ice sublimation from the nucleus. Desorption is enhanced by the interparticle collisions which take place near perihelion as the individual particles in the swarm move from above to below the median plane of the swarm orbit. Collisions also bring about the emission of light and the release of gases. Sublimation increases as the temperature of the nucleus increases. Meteoroid streams can be formed by the ejection of particles from the nucleus by gas pressure or by the fragmentation of swarm particles.

## Phenomena

The problematic phenomena are as follows. The coma generally contracts as the comet approaches the Sun, roughly in proportion to its heliocentric distance, and then expands again as it recedes. A swarm would do this. However, the observation is much more difficult to explain with an icy nucleus. The sublimation of the different icy species would have to have an unusual temperature dependence to fit the observations.

Some comets engender intrinsic forces which change their periods, this change being non-random and continuing from one orbit to the next for tens of years. The periods can both increase and decrease. Lyttleton explains how perihelion collisions in a swarm can result in a loss of orbital energy and an acceleration of the mean motion. But how can the energy of the swarm increase and the period decrease? With a rotating icy nucleus (and what is not rotating in the Solar System?) any delay in the sublimation process would produce a jet force with a component normal to the solar direction and thus accelerate or decelerate the orbital motion, depending on the sense of rotation.

Comets are seen to split up into separate components which move apart with relative velocities of a few m s $^{-1}$ . Asymmetrical jet action could spin-up the icy nucleus until it breaks up under the action of centrifugal force. It is difficult to envisage how swarms can split up.

Some comets undergo outbursts, sudden increases in brightness which can be as large as a hundred-fold. In

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the case of comet Schwassmann-Wachmann I the occurrence of these bears no temporal association with perihelion passage, as would be expected if the comet was a single swarm of particles. An *ad hoc* increase in the number of swarms in an individual comet helps, but goes against the whole tenet of Occam's argument. Meteor studies indicate that Encke has made thousands of orbits around the Sun and this gives Encke a recent inner Solar System lifetime of at least 10,000 years. Long period comets are expected to have lifetimes easily up to ten times this value. The high surface area to mass ratio of the swarm comet would make it a large collision target for the meteoroids in the Solar System dust cloud and collisions with these particles would slowly dissipate the swarm. Also a hierarchy of particle sizes would lead to a conspicuous spreading of the swarm along its orbit over 10,000–100,000 years under the action of the Poynting–Robertson effect. Swarm comets would thus have shorter lives than icy nucleus comets—possibly too short to agree with meteor stream data.

Some Sun-grazing comets actually pass through the solar corona, and the dusty insulating layers surrounding an icy nucleus seem to be required to enable the comet to withstand the intense heat. Swarm particles would vapourise. Even though recondensation is possible, the gas content and thus cometary activity would be much reduced.

### Arguments

These are some of the arguments that crop up in the debate—a debate which is not without its fair share of invective. For example, Whipple (*op. cit.*) dismisses the swarm hypothesis as 'totally unsatisfactory' and O'Dell (*The Study of Comets* part 2, NASA S.P. 393, 591; 1976) describes the swarm and nucleus models as 'the inconsistent and the unavoidable'. Lyttleton in his paper calls the icy nucleus model an 'invalid unacceptable hypothesis' to which has been added 'an elaborate set of ad hoc assumptions to try and escape the difficulties that the initial conjecture itself entails', a model which is 'ruled out at once by the principle due to Occam'. Before introducing the icy nucleus as a hypothesis 'it would be necessary to show with scientific certainty that specific cometary phenomena exist that are incapable of explanation by already available hypotheses'. In other words dust swarm out before icy nucleus in. But why? Surely hypotheses must be judged on their usefulness and not their historicity. Both can be subjected to the scientific rigour of checking and

## A liquid permanent magnet?

from P. V. E. McClintock

ACCORDING to A. J. Leggett (this issue of *Nature*, page 585) there is reason to believe that the A-phase of superfluid  $^3\text{He}$  may display ferromagnetic properties. An isolated sample of the liquid would thus be surrounded by its own spontaneously created magnetic field in much the same way as a conventional steel permanent magnet, although the physical origins of this magnetism would be rather difficult.

The onset of superfluidity (below a temperature of 0.0026 K) in liquid  $^3\text{He}$  is associated with the atoms forming themselves into pairs, each of which can be regarded as being in many ways rather like a giant diatomic molecule, with the two atoms orbiting around each other. A bulk sample of the liquid tries to arrange itself such that the angular momentum associated with each pair lies in the same direction, known as the *l* direction (giving rise to intriguing—and, as yet, unresolved—questions about the possible existence and magnitude of an intrinsic, macroscopic angular momentum for the liquid as a whole). In the A-phase the nuclei of the atoms in a pair are orientated parallel to each other and, because each nucleus carries a feeble magnetic moment, this might seem at first sight to offer the possibility of bulk ferromagnetic properties. It is known, however, that even in a very weak magnetic field the pairs of nuclei tend to align themselves in such a way that just as many lie anti-parallel to the field as are parallel to it. One can be confident, therefore, that the liquid will not develop any intrinsic magnetic field through spontaneous ordering of the nuclei. What Leggett has done is to point to the possible existence of an entirely different mechanism in which it is the electrons of the  $^3\text{He}$  atoms that might be able to produce magnetic effects.

It is well known that in a rotating diatomic molecule the electron shells tend to 'slip' a bit: the atoms forming the molecules do not orbit each other like rigid spheres. Thus, a little of the rotational angular momentum of a molecule gets transferred to the electrons around each of its two constituent atoms, producing electrical currents which in turn give rise to magnetic fields. For a conventional diatomic gas, however, the net magnetic field resulting from this phenomenon averages to zero because all the molecules are orientated at random relative to each other. (The effect may still be observed, though, from the broadening of nuclear magnetic resonance lines resulting from the randomly modified local magnetic field in which each nucleus finds itself.)

For  $^3\text{He-A}$ , on the other hand, the 'molecules' form a highly ordered system, so that the magnetic contributions of different pairs will reinforce each other, producing a macroscopic magnetic field whose direction will depend on that of the *l* vector. Leggett estimates the magnitude of this field, and he reaches the conclusion that it should be detectable in a suitably designed experiment.

If superfluid  $^3\text{He-A}$  is really ferromagnetic—and, on the basis of the simple 'giant diatomic molecule' model of the pairing, this conclusion seems to be almost inescapable—there will, as Leggett points out, be a number of interesting implications. Not least is the fact that the superfluid would apparently have been found endowed with yet another form of uniqueness: as being the only ferromagnetic liquid known in nature.

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improvement. To quote Leggett: 'it is not permissible to go on copying the same assumptions from one publication to the next, while simply ignoring either the contrary evidence or the lack of positive evidence'. So the adherents of the swarm hypothesis must investigate explicitly how a swarm can accelerate as well as decelerate in its orbit, how it can split up, produce outbursts, and how it can exist as a specific entity for  $10^4$  to  $10^5$  years. Those who favour the icy nucleus must again carefully investigate processes which can produce the

observed variation of coma size with heliocentric distance, the asymmetry of coma brightness, and the sometimes quickly varying spatial and temporal light condensations in it. But how can we satisfy Lyttleton?

He predicts that there is little possibility of this argument being resolved 'short of some startling optical development useable from Earth, or that the Earth should run directly into a long-period comet or a successful cometary mission has been carried out with equipment capable of finding the nucleus if it is there'. The second of these points

could, however, have been satisfied. Comets are not rare and Earth must have run into many in its lifetime. Brown and Hughes (*Nature* **268**, 521; 1977) found that a small  $5 \times 10^{10}$ -g comet hits Earth on average about every 2,000–4,000 years. Now swarm nuclei have cross-sectional areas about 60 times that of the Earth. An Earth-swarm impact would be equivalent to an intense and very short lived ( $\sim 1$  h) meteor shower. Unfortunately this would be indistinguishable from the phenomena produced by the passage of the Earth through an active new meteor stream. On the other hand, an impact between Earth and an icy nucleus comet would produce a large explosion low in the atmosphere or at ground level; Brown and Hughes proposed the Tunguska event as a strong candidate for just this type of impact. Unfortunately the surface evidence of such a cometary impact would be wiped out by erosion and the regrowth of vegetation after a relatively short time ( $\sim 100$  years). So we are lucky that Tunguska occurred only 70 years ago and is still evident. Older impacts have long since been erased. Needless to say, to propose that Tunguska was probably caused by a cometary impact is a far cry from regarding the Tunguska event as definite proof that comets have small dirty snowball nuclei.

I think the evidence is strongly balanced in favour of the icy nucleus model but it must always be remembered that it is only a model, not established fact. We still are far from knowing what a comet is for certain.

## Gel motility

from Dennis Bray

LIKE the electron, the cell shows a different face according to how it is treated; at least, that is the conclusion to be drawn from recent papers on cell movement. Even the intuitive picture of the inside of the cell seems to depend on the techniques used. To the biochemist for example, who isolates actin and myosin from various tissues, the cytoplasm appears to be full of miniature muscles. Membrane-anchored actin filaments slide against short myosin segments and in this way generate cell extension, membrane flow and intracellular movements. This 'biochemical' view has the unique advantage that it gives immediately the molecular details of force generation, but the overall picture is very blurred. How the small contractile elements are to be distributed and coordinated within

the cell is problematic and left largely to the imagination. By contrast, the 'cytoskeletal' view—which has been engendered mainly by the use of fluorescent antisera—shows the position of the fibrillar proteins directly. Here the cell is a complex basketwork of actin cables, microtubules and  $100 \text{ \AA}$  filaments, sometimes linked together in a geometrically precise fashion. The relationship of the different proteins to each other can be seen in gross terms, but so far it has not been possible to go down to molecular dimensions. Moreover, this picture has a distinctly inflexible quality and movement with such accessories calls to mind the grudging articulation of a child's construction set.

Most recently a number of investigators have, at least by implication, returned to what might be called the cytoplasmic or 'amoebic' view. They see the cytoplasm as a substance that can flow or set on demand and thus produce within the confines of the membrane the means of locomotion. The experimental basis of such work is the cell-free extract that can either move or change its consistency in a meaningful way. This was first shown to be possible in an experiment of Allen and coworkers (*Nature* **187**, 896; 1960), and a major part of the contemporary work is a direct descendant of this. As summarised by D. L. Taylor (*Cell Motility, Cold Spring Harbor* 797, 1976), the cytoplasm of free-living giant amoebae may be isolated into various test solutions where it responds according to their composition. Without free calcium the cytoplasm is immotile and lacks birefringence while the addition of as little as  $7 \times 10^{-7} \mu\text{M}$  calcium will cause contraction. Cells ruptured in threshold concentrations of calcium will extrude cytoplasmic 'flares' very like pseudopodia without their membranes. These transitions have been related to changes occurring within the intact amoeba and, in turn, there are indications that they depend on the state of aggregation of the cytoplasmic actin. The latter feature has been shown very clearly in recent work on the slime mould *Physarum* in which, as in amoeba, the rapidly streaming cytoplasm has two distinct phases. Isenberg and Wohlfarth-Bottermann (*Cell Tiss. Res.* **173**, 495; 1976), examined the droplets obtained by puncturing the organism which are at first sol-like and then change in consistency and mechanical strength to produce a gel. The drops were allowed to spread on the surface of small aliquots of buffer and then caught on an electron-microscope grid. Under the right conditions, within 5 minutes or so, a dramatic increase

occurred in the numbers of actin filaments concomitant with the maturation of the droplet: strong evidence that actin polymerisation is involved in the sol to gel transformation.

Another lineage of experiments, arising from a different initial observation but converging to a closely analogous position, concerns motile extracts of giant amoeba, acanthamoeba, slime moulds, sea urchin eggs, macrophages, leukaemic cells and HeLa cells. These systems were recently reviewed by Hitchcock (*J. Cell Biol.* **74**, 1; 1977), to whom the interested reader is referred. These extracts resemble the isolated amoeba cytoplasm in many of their properties but since they involve a number of purification steps they are—in spirit if not in simplicity—considered as biochemical preparations. They share an initial extraction into ice-cold buffers lacking calcium and containing ATP, and most of them use either sucrose or glycerol. They all show a temperature-induced gelation and they all have as their major component actin.

Beyond this the differences are many and somewhat confusing. Compare, for example, a recent paper on *Dictyostelium* (*J. Cell Biol.* **74**, 901; 1977), with one on cultured HeLa cells (*J. Cell Biol.* **75**, 95; 1977). One uses sucrose and a detergent in the extracting solution, the other a simple buffer; one extract will contract after gelation, the other will not; only a handful of the 20 or so proteins present in the extracts are obviously comparable, and so on. The burden to the casual reader is not made lighter by the zeal with which the workers in this field publish the minutiae of their results and their papers are characterised by the number and complexity of tables of ionic effects and figures showing protein composition. If there are general conclusions to be drawn from the comparison of various systems, the authors do not make them easy to find.

This is not a trivial point because it is possible to become data-drunk and confuse the formal similarity between the various observations for a common mechanistic basis. One can finish with the belief that the patently major cytoplasmic streaming and sol-gel transformation of a free-living giant amoeba is true of a vertebrate tissue cell: it may be so; but it has not yet been shown. Most importantly, the fact that these various sources can all yield protein gels is not evidence of a common physiology. Most proteins will form a gel if they are sufficiently concentrated—including muscle F-actin left in a refrigerator for 2 weeks—and their consistency and mechanical properties will inevitably depend on temperature



and ionic composition. No doubt there are gels and gels; but techniques are not available to make subtle distinctions. The usual test of how an extract falls out of an inverted tube is no more scientific than the criterion advanced by Fats Waller: that it must be jelly 'cause jam don't shake like that!

To be fair, the investigators are aware of these problems and make every attempt to relate the phenomena they are studying to cellular physiology. Already there are encouraging signs, such as that the contractile properties of a macrophage extract depend on the phagocytic activity of the cells (Stossel & Hartwig, *J. Cell Biol.* **68**, 602; 1976), and that cytochalasin B has a pronounced effect on the properties of some gel systems (Weihsing *J. Cell Biol.* **71**, 303; 1976; Hartwig & Stossel *J. Cell Biol.* **71**, 295; 1976). One can hope that the motile gels will be further dissected and even that they can reconcile the presently diverse views of cellular locomotion. We are so spoiled by the example of striated muscle—where the whole range of movement, from macroscopic changes in length down to molecular or even submolecular changes, can be linked in an unbroken series of logical steps—that nothing less will do. □

## Relevance of parasitology

from F. E. G. Cox

The British Society for Parasitology autumn meeting on The Relevance of Parasitology to Human Welfare Today was held in London on 28 October, 1977.

THERE is no doubt that parasitology has taken off, but it is germane to ask if some of it may actually have gone into orbit. It is, therefore, essential for parasitologists to get their feet back onto the ground and that is what this meeting was about. In the morning B. O. L. Duke (World Health Organisation (WHO), Geneva) spoke about medical aspects of parasitology, R. B. Griffiths (Food and Agriculture Organisation, Rome) about veterinary aspects and P. T. Haskell (Centre for Overseas Pest Research, London) about agricultural aspects. In the afternoon two invited speakers commented on each of these aspects and introduced specific and general discussion.

The morning's speakers set the stage,

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summarising the main areas of interest to and reiterating the parasitological problems of the developing world. The WHO Special Programme (*News and Views* **262**, 85; 1976) was outlined, but there can be few parasitologists who do not know what the questions are and no real attempt was made to provide any answers. The Special Programme is arguably one of the most important events in the history of parasitology and it would be a pity if it deflected attention from parasites which are not of medical importance. Griffiths left no doubt that diseases such as fascioliasis, trypanosomiasis and theileriasis maintain a stranglehold on meat production in the tropics, and singled out ticks as the major vectors of the diseases of domesticated animals. Considerable progress has been made in the control of these diseases—new and effective anti-trematode and cestode drugs such as Praziquantel are now available and successful immunisation of cattle against east coast fever has been achieved. Less dramatically it has been shown that larval stages of tapeworms which are not infective to man can be differentiated from those that are, making it possible to save vast numbers of carcasses. It may be that the amount of meat saved as a result of parasitological research is not much by western standards, but it is a major contribution towards raising the standard of living in the developing world and supporting the increasing population which will result from WHO activities.

Agricultural improvements are also necessary for the future of the developing world and there is evidence that the problems are not insoluble although insecticides will remain the major tool for many years to come. There is, however, much more that needs to be known about tropical ecosystems before it becomes possible to modify local or devise new agricultural methods, for it is now clear that the transplantation of western ideas and techniques creates new problems as well as solving some of the existing ones. In particular, the importance of plant nematodes is only just being understood (J. Bridge, Imperial College, London), and the differences between their activities in tropical and temperate lands may be immense. Land use in the tropics, especially Africa, is a perennial topic for discussion and W. E. Ormerod (London School of Hygiene and Tropical Medicine) drew attention to the limitations that soil and rain place on land use (see also Ormerod *Science* **191**, 815; 1976). The use of indigenous animals as sources of food was discussed, but possible parasitological problems were not considered.

One of the major sources of disquiet

discussed was the lack of any real future for drugs against parasitic diseases. J. F. Ryley (ICI Ltd, Alderly Park) pointed out that the development of any drug was now prohibitively expensive, mainly because of the regulations embracing such developments. The WHO also laid down conditions which effectively prevented many pharmaceutical firms from co-operating in joint ventures. Participants at the meeting were pleased to learn that these conditions are to be changed.

Another source of disquiet was the lack of career prospects for young parasitologists. Graduate unemployment is a world-wide problem and developing countries naturally wish to employ nationals in WHO and other schemes. The disenchantment of young people who have a real interest in parasitology and its direct application but who find that they cannot work where they feel they are needed is real. G. S. Nelson (London School of Hygiene and Tropical Medicine) drew attention to the achievements of parasitologists in the past and the important discoveries made by Britons in this field. It may well be that, in the past, devoted parasitologists working in less than optimal conditions took the assistance of local staff for granted. Perhaps the great discoveries yet to come will be made by local parasitologists and young Americans and Europeans will be better employed as their highly skilled technicians. It takes courage and real devotion to do the work and receive none of the glory, but the great achievements listed by Nelson were those of men who thought more about sick Africans than the number of papers published. □

## Two-photon ionisation interference effects

from Peter Knight

AN atom in a moderately intense laser field can ionise by absorbing more than one photon, and multiphoton ionisation is a very active subject at the moment. For example, it was hoped that the multiphoton ionisation of an alkali could provide, by courtesy of the spin-orbit interaction, a useful source of spin-polarised electrons. Preliminary experiments were performed at several laser wavelengths on the two-photon non-resonant ionisation of atomic caesium ( $\text{Cs} + 2h\nu \rightarrow \text{Cs}^+ + e$ ) by Van der Wiel and

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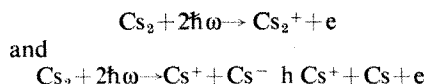
Granneman (*J. Phys.* **B8**, 1617; 1975). Much to everyone's surprise large discrepancies, of up to four orders of magnitude, were found between experiment and until then currently accepted theory. A predicted deep minimum of 2.6 eV photon energy in the two-photon ionisation curve arising from cancellation of 6p and 7p intermediate state contributions is entirely absent in the experimental data. Three years later, after much theoretical and experimental work, the problem remains, with no sign of a successful explanation. Similar paradoxical situations exist in the multiphoton ionisation of other atoms.

The experimental two-photon ionisation rates,  $P_2$ , are measured away from intermediate atomic resonances and at low enough laser intensities  $I$  to avoid saturation. Then the two-photon ionisation generalised crosssection  $\sigma_2$  at laser frequency  $\omega$  can be defined through  $P_2 = \sigma_2 \langle I^2 \rangle$  and  $\sigma_2$  is described by the second order perturbation result

$$\sigma_2 \propto \left[ \sum_n \frac{\langle f|r|n \rangle \langle n|r|g \rangle}{\omega_n - \omega} \right]^2$$

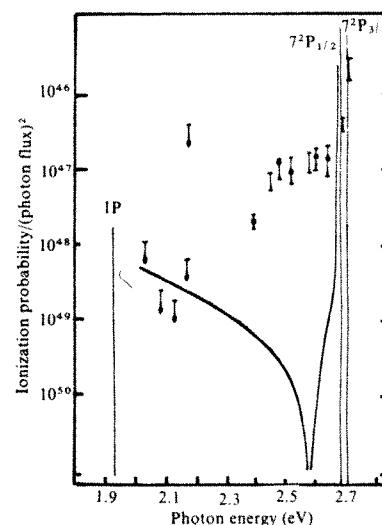
The atom is presumed to start in some initial state  $|g\rangle$ , end up in a continuum state  $|f\rangle$  and we need to sum all intermediate states  $|n\rangle$  of energy  $\hbar\omega_n$ . Often, as here in Cs at 2.6 eV, there is destructive interference amongst the terms in the sum: all signs are equal except energy denominators and  $\sigma_2$  should exhibit a deep minimum sometimes called a Fano or Cooper minimum. Problems facing the theorist involve finding accurate (although necessarily approximate) values for atomic wavefunctions using quantum defect, model potential or other methods and then to perform the summations. The existence and properties of the minimum have been reinvestigated by Teague *et al.* (*Phys. Rev.* **A14**, 1057; 1976) again assuming the validity of second order perturbation theory. They find the position of the minimum is insensitive to the choice of wavefunctions. There is some disagreement between theoretical results obtained by different methods, but they all differ very much more from experiment than from each other.

The original experiments of Van der Wiel *et al.* were performed using the nine argon-ion laser wavelengths available, and were calibrated by normalisation to a known one-photon cross section measured using frequency doubled laser light. The quadratic intensity dependence of  $P_2$  (and therefore the absence of saturation) was checked using neutral density filters. Since it is only a two-photon process, photon statistics of the laser light are relatively unimportant (they can contribute a factor of two at most). One problem is the contribution dimers make to the ion signal by processes such as



Relatively few dimers can provide a larger count rate than the numerically superior free atoms because they are resonantly enhanced and have a large density of resonant states. This molecular effect potentially can fill in the experimental minimum, but Van der Wiel and colleagues at FOM have studied these processes in detail (Granneman *et al.* *J. Phys.* **B9**, 865; 1976; Klewer *et al.* *J. Phys.* **B10**, 2809; 1977) and believe they have the dimers under control and that the discrepancy is not due to molecular background. Another possibility was that the minimum lies below the lowest energy available from an argon ion laser. This possibility seems now to have been eliminated (Klewer *et al.* *J. Phys.* **B10**, L243; 1977) by using an argon-ion pumped Rhodamine 6G dye laser to study two-photon ionisation at photon energies from 2.2 eV to near the two-photon threshold at 1.95 eV. Provided the temperature is raised sufficiently to remove dimers no detectable atomic two-photon ionisation remained in the wavelength at the (lower) intensities available from their dye laser. The data from all the wavelengths is compared with the best current theory in Fig. 1. In the same paper the ratio of the ion count rates obtained from two-photon ionisation of Cs atoms using linearly and circularly polarised light at the nine argon-ion wavelengths were measured. Again there is disagreement with theory, with the experimental ratio ( $\sigma_2(\text{circ})/\sigma_2(\text{lin})$ ) lying significantly below the 'best' values of Teague *et al.* except close to a  $7P_{1/2}$  intermediate resonance.

So we are left with a considerable problem. The approximate position of the Cooper minimum in the two-photon ionisation is determined by only a few matrix elements corresponding to transitions to nearby levels. Other levels shift the minimum by only a small amount.



**Fig. 1** Two-photon ionisation probability of Cs as a function of photon energy.  $\circ$ , Granneman and Van der Wiel (*J. Phys.* **B8**, 1617; 1975);  $\bullet$ , Klewer *et al.* (*J. Phys.* **B10**, L243; 1977).  $\dagger$ , upper limit found in the same ref.; full curve, Teague *et al.* (*Phys. Rev.* **A14**, 1057; 1976) theory. (Taken from Klewer *et al.*)

It would require a change in sign of either the matrix elements  $\langle 6p|r|6s \rangle$  or  $\langle 7p|r|6s \rangle$  for the deep minimum not to occur. Obviously more theoretical work on the calculation of  $\sigma_2$  is needed. Even the validity of second-order perturbation theory to describe the two-photon ionisation has been investigated, by Crance and Feneuille (*Phys. Rev. A*, in the press) who studied the time development of the pulsed photoionisation using a model introduced by Armstrong, Beers and Feneuille (*Phys. Rev.* **A12**, 1903; 1975). They have found some effects due to the finite pulse length, but their model is a little idealised. More experimental work is needed and this is being undertaken in various laboratories. Looking for a minimum is hard when background molecules are only too eager to fill the sought-for trough and future experiments will need to pay special attention to this problem.  $\square$



## A hundred years ago

DR. VOHL, of Cologne, has adopted an ingenious method of determining the impurities in the Rhine, which consists in analysing the boiler incrustations of the river steamers, as well as the concentrated residues remaining in the boilers after passing over a certain distance. By this means he has detected the presence of a large amount of arsenious acid in the river water—resulting chiefly from the aniline and dyeing establishments—as well as other poisonous substances. An unusually

high percentage of phosphoric acid showed that the sea was daily absorbing vast quantities of the most valuable fertilising material from the soil of Germany.

ANOTHER sitting of the enlarged Council of the Observatory of Paris was held on December 9. The councillors passed a resolution for an increase of the salary of the astronomers and auxiliary astronomers, the maximum pay of the former to be 10,000 francs instead of 8,000, and of the second 7,000 instead of 6,000. They propose to the Government to place the appointment of the director of the establishment partly in the hands of the Academy of Sciences and partly in the hands of the Council, the Minister to have only the privilege to choose between both presentations. From *Nature* 17, 13 December, 131; 1877.



THE lunar geological formations known collectively as the Fra Mauro Formation have long been thought, by most students of the Moon, to be deposits of ejecta from the basin of Mare Imbrium. When, in 1971, Apollo 14 astronauts surveyed an area not far from the crater Fra Mauro itself, it was hoped that new light would be thrown on this old conjecture.

The *US Geological Professional Paper 880*, US Geological Survey, 1977 examines merely a part of the apposite evidence: that relating, principally, to the astronauts' *in situ* findings and their hand-held camera records. Accounts of the terrain characteristics and of numerous rock blocks feature prominently in the description. The rock blocks range up to several metres in size. They are all breccias thought to have been produced by the welding of impact-generated fragments. The photogeological analyses of these rocks cover discussions of their structures and descriptions of their clasts. One block of rock has a probable clast, 1.7 m in length, in which other clasts are found and which themselves contain yet other probable, small clasts: this indicates successive and discrete stages in the evolution of this particular block.

The photointerpretation is supplemented by the astronauts' own field descriptions of the samples; and the publication includes coloured geological maps, charts, and panoramic photomosaics to assist the reader with the identification of a sample's relative position and environs.

Good descriptions are presented of three kinds of rock fillet—an accumulation of fine-grained material (see photo) making contact with a co-

## Surveying the Moon

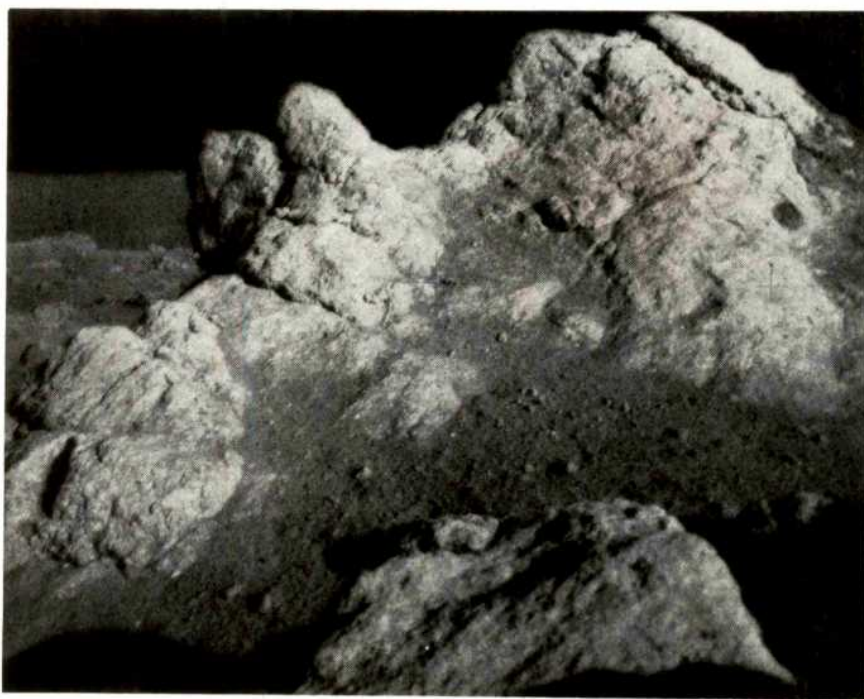
from G. Fielder

herent block or rock—and of other features which point to physical processes on the lunar surface. Among these features, the lunar grid (lineament) system is put in perspective when the authors argue for the correlation of small-scale (centimetre to metre lengths) lineament trends with trends mapped on a large scale and dismiss the proposition that lighting

can have an all-important bearing on the apparent directions of most lineaments.

Although the matrix materials of the rocks studied in detail on this fine collection of photographs are frequently referred to, in the text, as of probable Imbrium origin, the Fra Mauro Formation is said (without qualification), in the Summary of Conclusions, to consist of ejecta from the Imbrium basin! □

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One of three kinds of lunar fillet: lunar regolith fines have been scattered on to this gently sloping, structured rock face and have settled in shallow depressions to produce a 'low angle fillet.' (NASA photo AS-14-68-9450)

## Benioff splitting

from Peter J. Smith

BENIOFF ZONES are intimately associated with earthquakes. Indeed, earthquakes provide the best means of delineating such zones, indicating with startling clarity how moving oceanic lithosphere bends downwards beneath appropriate continental edges and descends at an angle of about 45° until it is ultimately assimilated into the mantle. But whereas it is customary to see a scattered pattern of earthquakes defining the outlines of a descending plate, more recent observation has indicated the presence in some

cases of a fine structure of seismicity which may throw light on at least one of the processes by which Benioff zone earthquakes are produced.

A nice example is provided by Engdahl and Scholz (*Geophys. Res. Lett.* **4**, 473; 1977), who have analysed the distribution of foci from all earthquakes with magnitude greater than 3 occurring from July 1974 through February 1977 in the Adak region of the central Aleutians. The resulting plot shows that most of the shallow seismicity in the area occurs within the depth range 15–27 km and has a lateral spread of about 50 km in the direction of the horizontal component of plate motion (that is, viewed in side projection, looking end-on at the arc). Immediately below, the zone of seismicity then contracts to a lateral width of no more than 10–15 km. At a depth

of about 100 km, however, the seismicity splits into two distinct zones each of which has a lateral dimension of 10–15 km and is separated from the other by a practically earthquake-free region about 25 km wide. At a depth of about 175 km the two arms then come together again, forming a single zone of seismicity down to the deepest earthquakes at about 270 km.

This is not the first known example of Benioff splitting. Sykes (*J. geophys. Res.* **71**, 2981; 1966) observed a separation of about 30 km in the pattern of intermediate earthquakes beneath the Kurile arc more than a decade ago; and more recently Veith (thesis, Southern Methodist University, Texas, 1974) and Stauder and Mualchin (*J. geophys. Res.* **81**, 297; 1976) have investigated the Kurile case in greater detail. Umino and Hasegawa (*Zisin* **28**,

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125; 1975) have also demonstrated the existence of splitting beneath Japan. In each case the earthquakes in the upper arm of the split indicate down-dip compression whereas those in the lower arm show down-dip tension.

But is such splitting typical or atypical of Benioff zones? And either way, what causes splitting and the associated difference in focal mechanism? The answer to the second question may well suggest an answer to the first. One possible explanation is that the two arms of seismicity represent two closely associated, but distinct, descending slabs. But although this possibility is not inconsistent with the Adak splitting, it cannot, according to Engdahl and Scholz, account for the Japanese and Kurile examples; nor can it explain the difference in focal mechanisms in any of the three cases. Then it is possible, as suggested by Veith, that splitting is related to the olivine-spinel phase transition, although Engdahl and Scholz make no comment at all on that.

What they do, however, is to propose, following Isacks and Barazangi (in *Island Arcs, Deep Sea Trenches, and Back-Arc Basins*, edit. by Talwani M. & Pitman W. C., American Geophysical Union, 1977), that splitting is due to deformation of the lithosphere during subduction, and to follow up the proposal by constructing a model which seems to account for the observation. The basis of the model is the fact that subducting lithosphere bends at shallow depths but straightens out again as it moves at an angle towards the mantle. Whatever the cause of these changes, the 'unbending' must produce stresses in the descending slab; and Engdahl and Scholz are able to show with little difficulty that, at the observed depths of splitting, an 80-km thick lithosphere has a 22 km thick elastic core with down-dip compression on its upper surface and down-dip tension on its lower surface.

Thus the double zone of seismicity can be explained simply as the result of the release of unbending stresses above and below an elastic core within the lithospheric plate. Moreover, as the plate descends into hotter and hotter regions the elastic core will get thinner and ultimately disappear altogether, which explains why splitting occurs only over a limited depth range below the initial bending of the lithosphere and does not persist to all depths. Since unbending must occur in most, if not all, descending plates, it follows that splitting must be typical of Benioff zones. Failure to observe it in any given case presumably therefore reflects only insufficiently detailed observation and analysis of earthquake foci. □

## Jeffreys Lecturer links Sun and weather

from John Gribbin

IN this year's Harold Jeffreys Lecture of the Royal Astronomical Society, presented on November 11, J. W. King of the Appleton Laboratory reported the latest work of his team on 'The Influence of Solar Phenomena on Weather and Climate.' Over the past few years, King and his colleagues have received a rough ride from some quarters for their failure to explain the physical basis of the links they have found between Sun and weather; commenting here that the situation is no different from that in climatology, where we know the Earth's climate does change without being able to say exactly why it changes, King went on to present convincing evidence of the reality of the link on timescales down to days, and threw out the challenge to both solar physicists and meteorologists to join the Appleton team in investigating these phenomena further.

To most of the audience, and most readers of *Nature*, the debate about solar influences on weather over the sunspot cycle and longer periods must already be familiar. But the new work from the Appleton Laboratory concentrates instead on much shorter term influences, associated with the roughly 27.5 d rotation period of the Sun. The 'signature' of this rotation shows up clearly in such meteorological parameters as the height of the 500 mbar pressure level in the atmosphere, as well as influencing ionospheric properties. In some danger of numbing his audience with overkill at times, King hammered home the reality of these links with a wealth of data, the most intriguing of which showed clear geographical variations of the magnitude and even sign of the atmospheric changes produced by the Sun. Small wonder, then, that global averages over long periods show much less indication of any solar influence on weather!

The solar influences are particularly strong in a region above the Atlantic just west of the British Isles, which makes this link between Sun and weather especially important for residents of those islands. And if these small variations over the solar rotation cycle can affect weather parameters, then it comes as no surprise to find specific larger events on the Sun—flares and so on—producing specific larger disturbances of the Earth's atmospheric systems.

Such a link has, in fact, been known for some 30 years, since the pioneering work of Duell and Duell (*Smithsonian Misc. Coll.* **110**, 8; 1948) but has only relatively recently become firmly

established (see, for example, Olson *Nature* **257**, 113; 1975). The Appleton team have now found that not only does a solar flare produce a disturbance of the atmosphere, commencing some four days after the flare, as shown by the work of Olson and others, but that the atmospheric disturbance is repeated at 32 and 60 days as well, one and two solar rotations later. In at least one case, an effect on the atmosphere is also found at -24 d, that is one solar rotation before the flare became apparent. So a specific region of the Sun which is involved in some disturbance can affect specific regions of the Earth's atmosphere over a period of 2-3 months—and, equally, the effects of solar activity which are disturbing the atmosphere today must be the integrated effects of several such past disturbances, which suggests that we should not find any simple relation between what the Sun is doing today and what the atmosphere does tomorrow.

King also described investigations of the longer run of data, going back for 100 years, available from UK Met. Office statistics, and these show a clear influence of the solar activity on occurrence of westerly weather patterns over the UK, that is, the weather coming from the region of the Atlantic where the solar influence is strong, and moving towards the British Isles. But this evidence was merely the icing on the cake of his presentation.

The challenge to meteorologists is clear, since these effects are certainly large enough that by ignoring them all the present general circulation computer models (GCMs) must be in error. Could this be why such models are notoriously unreliable if run for more than a few days ahead? And equally the challenge is there for the solar physicists to explain what is going on—with the incentive that the work becomes of direct importance and value now that the link with the weather is emerging.

So King closed by saying that the pathfinding stage of this Appleton Laboratory work is now at an end, with the establishment of the reality of the Sun-weather links. In this essentially interdisciplinary study, the time has come for collaboration between the Appleton group at the centre and the meteorologists and solar physicists on either side; 'I hope,' he said, 'that this lecture will mark a starting point in progress towards achieving practical benefits.' □

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## My Uncle Eustace—Maker of Rings (1885–1916)

A HAPPY solitary child, after  
his mother died so self contained  
and yet attached by magnet  
chain to Margaret the country girl  
who mothered him at Vicarage;  
his father never understood  
or tried to peel, excise  
the layers of his mind, beneath  
the courtesy, the grave concern  
so strange in one so young, one sensed  
always a sunlit glow, a pool  
of quiet stillness that no naval mine,  
no six-inch shell could shatter.

He passed, as far as clerical  
finances would permit, to prep., then  
boarding school, no niche  
seemed ready carved, a solitary boy  
puzzling to House and Form  
master and sixth form prefects, yet  
always just scraping through  
two from the bottom everywhere,  
cold shouldered, taunted, ragged  
he would endure quite unconcernedly, until  
one famous bully, older by two years  
provoked him ceaselessly and on one afternoon  
when batting on the Common, suddenly  
young Eustace threw down his bat and strolled  
slowly down pitch and felled  
braggart to turf with one fierce swing.  
Always, when this deed had sunk  
swiftly to groundswell, not merely left  
in peace but elevated to that slot reserved  
for the eccentric, rare, unique, original.

One confidant he found, old Bony Head  
the science master who could sense  
a mind attuned intuitively to select,  
assess causal relationships, communicating flows  
built up from pulsing waves in orbit,  
sure, symmetrical, cast in one mould, one unitary mass.

Astounded and affronted was his family,  
that hierarchy of patrons, chiefs,  
that network with edges pinned in Burma,  
Delhi, London when he gained  
a science scholarship to Trinity,  
a field, a slippery slope fit  
for no gentleman to tread and yet,  
with mother's tiny nest egg to augment,  
how could he be gainsayed?

Sheep-like he merged within the Cambridge  
landscape; who knew or cared  
exactly where his lodgings lay  
or whether he built up  
stable relationships? He never said  
how marvellous those days, how glorious the golden youths  
with whom he melted smoothly down in Hall  
or rowed in college eight.  
Always and only shone an inner light.

His tutor was non-plussed because  
over the wide field of chemistry, he barely  
could pass muster, might just expect  
to scrape a Third and yet in one small  
unexploited corner of the craft he delved  
and bored and quarried ceaselessly;  
the hetero-cyclic, alicyclic rings,  
those compounds linked from carbon, nitrogen,  
oxygen, phosphorus and halides, elements  
rare, of dubious and unestablished  
property and mass and controversial valency  
and metals of odd groups, forced them to bind,  
adhere in hoops and circles, cubes  
and boats and knotted cord-like  
thongs; these problems he devoured,  
consumed with every meal, by them  
he was possessed, their literature he absorbed  
in tiniest detail, their personalities,  
their history; as then there was no other specialist  
at hand, this bred a bright hot fury of debate  
whether he merited a First, a Third, a Pass.  
Faith won the day, though outwardly indifferent,  
or so he claimed, so long as he could scoop  
a bursary to study for two years at Heidelberg,  
the fountainhead and spring of all clear waters  
here in this smooth fluency in which  
his flimsy ship was launched, must sail  
and pitch and toss on ocean till it sank  
shipwrecked on rocks, or ran relentlessly  
to calm deep water harbour.

But Eustace never felt at home with Germans,  
yet from them learnt all that he could,  
kept secret much, ploughed down to dusk  
and thirty days a month, except  
for Sundays when he'd read and walk  
to early service in the English church;  
how, in those days, these days, those ever  
other days can one combine science  
with Christ? Well, Eustace tried and yet  
he'd never talk, explain, attempt to justify,  
but said 'it's part of me and always will be,  
so please don't push me further'. How much  
privat-docents, professors knew  
or cared or understood about his bench-work  
problematical. Much that he wrestled with  
he never spoke about, he sealed his notes  
tight locked within a safe. After two years  
his thesis was submitted, was so unique,  
outside all normal channels, unbelievable  
until one sub-professor delegated for two months  
repeated parts in the minutest detail  
and found each component and experiment  
immaculate, repeatable with all analyses  
confirmed in unimpeachable statistical  
integrity; in essence so non-plussed  
the academical establishment, so much at doubt,  
so acrimonious the conflict that at last  
his D.Phil. was awarded and they asked  
that he submit his name for hierarchical preferment.  
Eustace said 'NO' and coming home in nineteen  
ten found no response from British  
chemical grandees in any university.  
Instead, sensing in bone that school

or industry demanded volumes of life-blood he dared not lose and live, would dislocate all plans for studies targetted, he ferreted instead a newly mortared niche in red brick college of technology in Yorkshire. Here, simply was demanded three lectures weekly and all the space of evenings, week-ends and Long Vacations free to theorise, experiment and scribble round the clock.

All work, you say, no private life and so it might have been had he been able to forget those German years; within his blood-stream multiplied the germs of fear, of certainty of wars to come. Exactly numbered days and calibrated therms of energy he gave to Territorial Brigade of Heavy Artillery, became an adept at range-finding, the most professional of officers from whom Colonels of regulars bent down to claim advice. Upon parade he stood one rank beneath my father, who, since a boy-hood thirled to the Lovat Scouts was always army mad. I never knew another so assiduous at work, at drill. He breathed in Elgin ice clear air where grandfather was bank cashier but dad determined from the very day he learnt to read to mount two ladder rungs beyond his family, became Chartered Accountant, then set up in solitary practice here in Yorkshire, where his twin Fiona kept house for him and taught German and English in Ladies' Private School.

Enmeshed by such a pair, how could poor Eustace disentangle from the cords of fishing net? My Aunt Fiona was not outwardly clever at all, a quiet fair haired filly, she seldom spoke so that the Head judged her to be submissive, compliant and then finances being strained, conveniently forgot Fiona's name on the Promotion List and when her resignation came by the first post next morning, a Parents' Protest Committee quickly crystallised and threatened to remove their girls to local Grammar. So, my uncle equally became submissive, really in fact enjoyed his thralldom, as she controlled all family affairs and left his week-ends free for pure research and gunnery.

A scientist is judged by all he publishes, the volume and the quality, the range of the discoveries and theories that appear in journals of accredited repute, the details of the fields that he has ploughed, the implements and measures at the base of tower and palace and with what ease his syntheses were found repeatable and could be used as spring board for fresh thinking. As my uncle Eustace published *nothing*, his reputation faded by neglect and he was soon forgotten by the élite of academic chemistry.

One Sunday, after tea, both families still sitting round the cloth, we children, cousins, were dismissed to garden. Rain, lightning shattered down and we returned to the worse thunder of a family dispute. 'To Hell', my father shouted, 'with academic honour. "May science never be any damn use to anyone" is fine for younger sons of viscounts but not for you, young Eustace, you don't possess a bean and when war comes, what will you leave your wife and kids to live on, if we're killed, as very well we may be; why should they starve because you are too proud to patent, publish your really rare discoveries? Now, fetch them out this minute, there's a good lad and we'll discuss their possibilities.' Fiona sided, not with my father but with her husband, swore she'd work and could support them all. But Uncle Eustace, in sudden silence, strode to his study and thence bore back a pile of manuscripts. While we all watched, in silent awe, the two men there and then selected ten of his theses which my father slipped away, for processing by patent agent friend.

When nineteen fourteen broke, the Battery with other amateurs was very soon immersed in battle. For two whole years my uncle strove with howitzers and mortars. His easy skill soon earned promotion and he found himself a temporary major at Army Corps Headquarters but, odd man out as usual, this election served only to convey boredom, so he transferred to Flying Corps and brought to bear his ingenuity in coaxing speed, manoeuvrability, quick reflex action from his Sopwith Scouts, high above battlefields in dog fight and reconnaissance.

Your past will find you out, advance upon you and on one August afternoon, recalled to C.O.'s hut he was commanded to collect all gear as he was ordered back to Ministry in Whitehall where the back-room boys had delved among his patents and discovered there the basis for a new, intensely powerful explosive which might win the very war. My uncle, angry, overruled, strolled slowly across airfield to his plane, to bid good-bye, as to a favourite terrier dog and at that hour the German fliers dived from cloud to rake the 'drome with fierce machine gun fire. Thus, Uncle Eustace died.

A posthumous hero, a genius accorded after a death so quizzical, so individual, yet so much of the pattern he had set himself to follow: my thoughts, my words still after thirty years confused, ceaseless in motion change from day to day and still I cannot say why this should be or if a yet more glorious world might shine had Uncle Eustace lived, lined up with others of his tribe, with Moseley and Wilfrid Owen, Rupert Brooke, the roll is endless.



I can record twenty-five monographs  
in Transactions of Academies world-wide,  
his name on plaques, on chapel walls,  
columns and references in text books, scores  
of new compounds indexed, in this  
his family name. Even the Fifth Form schoolboy fails  
if he forgets what Eustace stands for,  
and, as for his patents, Dad was very soon  
besieged by major companies in Widnes,  
Delaware, Paris, Turin and Hamburg outbidding  
all to purchase royalties for synthesis  
of basic chemicals for fertilisers, textiles,  
dyestuffs, drugs. So Father waxed, became  
commander on a field he was well qualified  
to master, transferred his offices to London,  
was made director of ten companies, Lord Mayor  
and baronet.

Succeeding him, I feel  
a conflict of emotions; undeserving, why  
should I and all my family be circled round  
with so much satiety, delight, so many friends  
and joys of love and freedom, beauty, esteem and honour  
and all because of one young uncle who  
pursued his goals with such humility  
and silence and a quiet smile? And yet  
I never can forget that glow, that steady  
luminescence in his eye which I, as child  
divined and marvelled at, sitting without movement  
beside him on the window seat, those birds,  
those flowers, as are the stars unquenchable,  
eternal, so can I never understand  
how light of such a clarity can fade,  
what medium that I can comprehend  
could generate the power  
to drown and snuff it out?

Deric Bolton\*

*Deric Bolton was, until 1971, a chemist with Macfarlan Smith Ltd, Edinburgh. His four published collections of poetry include The Wild Uncharted Country (1973), a narrative sequence on life in the chemical industry.*

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## Three centuries of alcohol in the British diet

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*Alcoholic drinks were consumed in larger quantities in the eighteenth and nineteenth centuries than in the twentieth century, although there has been a recent increase from the historical low of 1930–60. Beer, spirits and wines once provided at least 2 MJ (nearly 500 kcal) per person per day compared with 0.67 MJ (160 kcal) in 1975, towards an average energy requirement of the total population little different from that needed now. Beer has always contributed most to the alcohol, energy and nutrient content of the diet, although its importance relative to spirits and wine has declined.*

THERE is much concern about the amounts of alcohol drunk in the United Kingdom<sup>1,2</sup>. It is believed that men and more particularly women and children are drinking more, resulting in increased prevalence of drunkenness<sup>3</sup>, driving under the influence of alcohol<sup>1,3</sup>, alcoholism<sup>4</sup>, liver cirrhosis<sup>4</sup>, and absence from work owing to excessive intake of alcohol<sup>5</sup>. It is, however, very difficult to obtain accurate information from individuals. Most, when asked, are likely to underestimate their consumption, while some, wishing to impress the interviewer, exaggerate their capacities.

A more objective assessment of overall trends can be obtained from HM Customs and Excise Department, which records, for tax purposes, the total amounts of beer, spirits and wines retained for consumption within the United Kingdom (except for the small amounts of beer and wine made in the home). Since 1955 the Ministry of Agriculture, Fisheries and Food has assessed the energy content of these drinks and compared them with the energy content of the national food supply<sup>6</sup>. The results are summarised in Table 1.

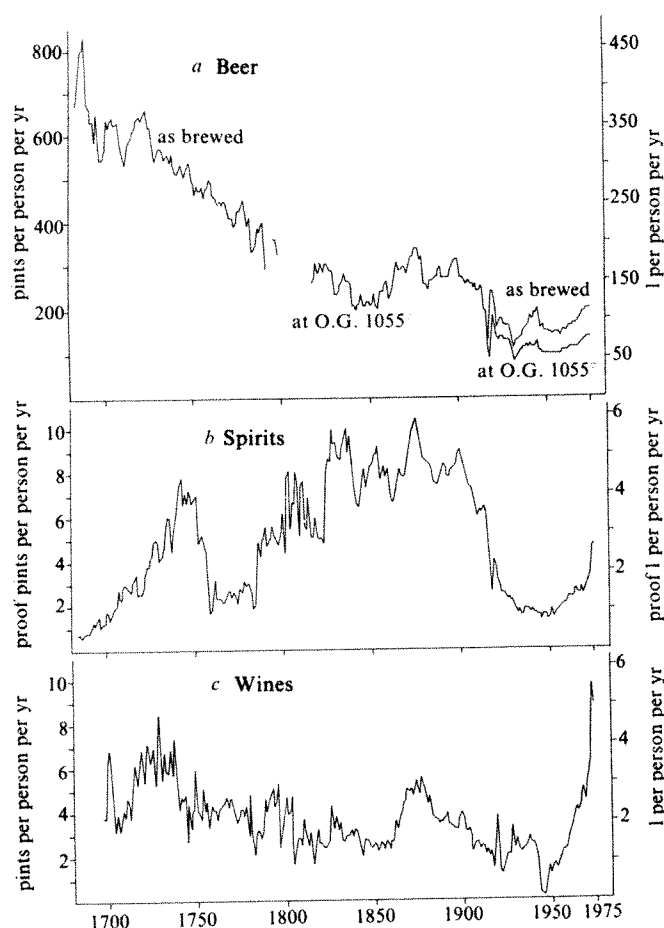
We wished to place these figures into historical perspective, for this rise in the consumption of alcoholic beverages started

from an historical low point. Customs and Excise records, with brief exceptions, have been collected since 1684 and are summarised in Fig. 1. Note that this figure is an average for the total population of the country, including young children who nowadays consume little or no alcohol. A discussion of separate trends for each beverage will be followed by a general discussion of the major factors which can affect consumption. Tables 2 and 3 list some of these.

### Beer

Ale has been drunk in England since Celtic times<sup>7</sup> and hopped beer since the fifteenth century, but it was not until 1643 that a beer duty was imposed to raise money for the Civil War. The earliest national records of consumption date from 1684, when the beerhouse was the centre of the working man's life. Many workers were paid at public houses, which also served as labour exchanges; if a man was unemployed he could receive credit if he was a regular customer<sup>8</sup>. Inns also served as centres of transport, courts and even small prisons, such as the White Lion at Southwark<sup>9</sup>. Entertainments such as cock fighting, bear baiting and prize fighting also took place in them. In the late seventeenth and early eighteenth centuries it was wiser to drink beer than the usually polluted drinking water. For example, Nottingham's water supply was drawn from the River Leen which also served as the main sewer of the town; fortunately Nottingham had one alehouse for every 80 inhabitants<sup>9</sup>.

Beer consumption reached a maximum of 832 pints per person per yr (or 2.3 pints per person per d) in 1689. Consumption dropped sharply when the duty was tripled (see Table 2) with partial recoveries after 1700 and 1712 when part of the tax was transferred to the constituent malt. Consumption then declined steadily until 1790 while the duty remained relatively static (as did the price: in London porter remained at 3 pence per quart until 1761 and 3½ pence until 1799 when it was increased to 4 pence<sup>9</sup>). There are no obvious reasons why consumption



**Fig. 1** Beer, spirits and wine available per person in the United Kingdom. *a*, Production figures for beer represent barrellage brewed by common brewers and brewing victuallers, corrected until 1830 to include private brewing. From 1684 to 1830 they are taken from Monckton<sup>23</sup>, from 1831 to 1936 from Wilson<sup>11</sup> (appendix F) and from 1937 to 1975 from the Annual Reports of HM Customs and Excise Department<sup>24</sup>. Volumes and populations refer to England, Scotland, Wales and Ireland up to 1922; afterwards Eire has been excluded. *b*, Values for spirits from 1684 to 1799 are from the Department of Inland Revenue<sup>24</sup>, from 1800 to 1935 from Wilson<sup>11</sup> and from 1936 to 1975 from the Brewers Statistical Handbook<sup>3</sup>. Volumes and populations refer to England only until 1713, and between 1718 and 1724 to England and Ireland. Thereafter they are for the United Kingdom. *c*, Values for wines from 1697 to 1900 are taken from Wilson<sup>11</sup> and from 1901 to 1975 from HM Customs and Excise<sup>24</sup>. Until 1787 they refer to wines imported into England, but subsequently are the amounts retained for home consumption in the whole United Kingdom (including Eire until 1922). The populations of the appropriate regions of the United Kingdom for decennial periods from 1684 to 1831 are from the Department of Inland Revenue<sup>25</sup> and thereafter from the Registrars General<sup>14</sup>.

decreased during this period, although from 1750 the enclosure of agricultural land and the initiation of the industrial revolution (which shifted population to the manufacturing towns) must have reduced home brewing<sup>10</sup> particularly in the North and Midlands. In the South shortages of fuel had a similar effect<sup>7</sup>. In 1791 a tripling of the malt duty resulted in another sharp fall in consumption. Records from here until 1880 are incomplete, for almost all those provided by the Brewing Victuallers' Association between 1791 and 1818 are lost and those between 1831 and 1880 have been derived from a mathematical conversion of malt to beer<sup>11</sup>, because beer itself was not taxed during this period, only malt and hops, and, from 1847, sugar. Consumption apparently increased as a result of the Beer House Act 1830 which allowed anyone who bought a 2-guinea licence to sell beer; 40,000 new public houses were opened within 5 yr (ref. 11).

In 1880 Gladstone introduced his free mash tun system allowing brewers to use carbohydrate sources other than malt, the duty being charged on the original gravity of the beer. This led to a decrease in price which, coupled with the nation's prosperity, caused consumption to rise to nearly 1 pint per person per d in the latter part of the nineteenth century. Thereafter consumption fell again, accelerated by an increase in duty in 1900, and reached its lowest recorded level in 1918. During World War I opening hours were restricted (see Table 3), and in 1917 production was reduced from 26 million to 10 million standard barrels and the gravity of the beer greatly reduced. The weakness and expense (after several increases in duty) resulted in a boycott of beer in many places. After the war consumption recovered, but then fell with the economic depression of the 1930s. World War II prompted a substantial increase in consumption, mainly because of the unavailability of spirits and wines, but demand was met by a reduction in gravity. Despite increases in duty, consumption remained stable after the war and rose from the late 1950s. Nevertheless the amount of beer available in the 1970s is considerably lower than it was 100 years ago.

A recent estimate put the amount of home-brewed beer produced from kits at 1.75 pints per person per yr.

Tax has been levied on cider for a few short periods only, and consumption is thus known for relatively few years. From 1756 to 1765 it ranged between 2.5 and 5 pints per person per yr; in 1820 and 1830 it was 2 pints but declined to little over 0.5 pints in some of the intervening years; and it enjoyed a brief increase to 4 pints at the end of World War I. The Cider Manufacturers Association have calculated cider consumption since 1946 as about 3 pints per person per yr until 1965, after which it rose rapidly to 5.8 pints in 1975<sup>3</sup>. Cider has been taxed since that year.

## Spirits

Before the Restoration the consumption of spirits was small, but after 1660 consumption, mainly of brandy, increased<sup>7</sup>. In 1688 William of Orange became king and encouraged the production of gin by issuing charters to divert the surplus English grain for its production; in 1690 imports of foreign spirits were prohibited. In Queen Anne's reign the monopoly privileges of the Worshipful Company of Distillers were cancelled; this led to the unlimited production of gin, much of poor quality, which was sold in the streets and hawked from door to door at 1 penny per pint. The steady increase in consumption was checked in 1729 by the introduction of retailer's licences costing £20 per yr and the prohibition of street hawking. Distillers retaliated with a flavourless, low quality spirit, not classed as gin (known as Parliamentary brandy). In 1733 the Act was repealed, and another introduced which limited the sale of spirits outside dwelling houses. This led to the development of the 'gin shops' typified in Hogarth's engraving 'Gin Lane'<sup>12</sup>. Thus gin and other crude spirits were both cheap and readily available at a

**Table 1** Increasing contribution of alcoholic drinks to the diet

Year	Energy from food MJ (kcal)	Beer (pints)	Spirits (proof pints)	Wine (pints)	Energy from alcoholic drinks (% of food energy)
1955	13.3 (3,170)	140.2	1.8	2.7	3.0
1958	13.3 (3,180)	138.2	2.0	3.2	3.0
1961	13.2 (3,150)	155.8	2.4	4.3	3.5
1964	13.2 (3,160)	160.3	2.7	5.5	3.7
1967	12.9 (3,080)	165.6	2.6	6.1	3.9
1970	13.0 (3,110)	178.6	2.8	6.6	4.1
1973	12.2 (3,040)	196.9	4.3	11.2	5.0
1976	12.3 (2,930)	209.0	5.1	12.0	5.7

The energy content of the food supplies relates to the total quantities available in the UK from agriculture and imports, less exports and non-food uses, and is expressed per person per d. The quantities of each alcoholic drink (per person per yr) are derived from Customs and Excise records, and their energy content includes any carbohydrate as well as the alcohol present. 1 litre = 1.76 pints.

Table 2 Major changes in duties charged on alcoholic drinks

Beer and constituents		Spirits		Imported wines		Index of wholesale prices <sup>24-27</sup> (1860 = 100)	
1643	Small beer 6d; strong beer 2/-	1684	2d-4d	1787	French 2/3d-3/1d	1680	55
1690	Small beer 1/6d; strong beer 6/6d	1751	1/- - 1/3d		Rhenish 3/1d	1700	80
1710	Small beer 1/4d; strong beer 5/-;	1783	5/10 $\frac{1}{2}$ d		Spanish 2/-	1750	70
	malt 6 $\frac{1}{2}$ d	1785	2/7 $\frac{1}{2}$ d		Portuguese 1/6d	1800	125
1791	Malt 1/7 $\frac{1}{2}$ d	1795	4/4 $\frac{1}{2}$ d		Hungarian 3/6d	1830	95
1831	Beer duty repealed	1825	7/-		French 7/2 $\frac{1}{2}$ d	1850	85
	malt 2/7d; hops 2d	1860	10/-	1825	Rhenish	1880	180
1850	Sugar 1/4d	1890	10/6d		Spanish	1900	135
1880	Beer duty reimposed 6/3d	1909	14/6d		Portuguese	1910	160
1900	Beer 7/9d	1918	30/-		Cape 2/5d	1920	315
1916	Beer 25/-	1919	50/-	1849	Colonial 2/9d	1930	220
1918	Beer 50/-	1920	72/6d	1860	Foreign 3/-	1940	265
1920	Beer 100/-	1943	154/-	1861	All light 1/9d; heavy 2/5d	1950	475
1931	Beer 134/-	1948	210/-	1920	Light 2/6d; heavy 6/-	1960	645
1948	Beer 178/-	1964	236/-	1927	Colonial light 2/-; heavy 4/-	1970	820
1959	Beer 110/-	1968	376/-		Foreign light 3/-; heavy 8/-		
1966	Beer 188/-	1973	308/-	1948	All light 25/-; heavy 50/-		
1973	Beer 138/-	1974	340/-	1958	Light 13/-; heavy 38/-		
1974	Beer 186/-	1976	492/-	1969	Light 32/2d; heavy 54/2d		
1975	Beer 273/-			1973	Light 17/7d; heavy 39/6d		
1976	Beer 317/-			1975	Light 53/6d; heavy 70/-		

12d (pence) = 1/- (shilling) = 5p (new pence). Beer duty is per barrel (163.6 l), malt and hops per bushel (36.4 l) and sugar per hundred-weight (50.8 kg). Spirits duty is per proof gallon, that is per gallon (4.5 l) with strength corrected to 100° proof or 49.276% alcohol by weight. Wine duty is per gallon.

time when the social conditions of the urban poor were extremely bad with little hope of escape other than intoxication<sup>13</sup>.

The Acts of 1751 and 1752 (Table 3) resulted in a dramatic decline in consumption and for the rest of the century consumption largely reflected impositions of duty.

The wild fluctuations of the next 20 yr may be partly due to a breakdown in the system by which spirits were taxed. Indeed it was not until 1824 that the figures of the Excise Department approximate to the real production<sup>14</sup>, and all estimates of production before this date must be regarded as conservative because of the amount of illicit distilling which was practised, particularly in Ireland and Scotland. Thereafter consumption was again influenced by duty and legislation, and religious opinion had further effects: for example Father Matthew's temperance mission in Ireland caused consumption there to drop by 23% between 1839 and 1845<sup>11</sup>. By the early 1870s, however, the equivalent of 4 ounces of proof spirit was consumed per week for every man, woman and child in the country; this was a time of great prosperity in the United Kingdom. Thereafter duty increases and trade depressions led to a steady decline.

In World War I, opening hours were curtailed, and the manufacture of spirits was then reduced on the orders of the food controller in 1917 to half that produced in 1916<sup>11</sup>. In 1918 consumption rose dramatically with the post-war celebrations in spite of increased duties, but then declined right through the economic depression and on into World War II when available grain was used for food rather than distillation. Consumption recovered after World War II despite increases in duty in 1947, until in 1964 a substantial increase in duty led to a stable consumption rate. In 1973 the duty was decreased and the increase in consumption has now continued in spite of several more tax increases.

## Wines

In the Middle Ages wine drinking was common in Britain and consumption was relatively high. The dissolution of the monasteries led to the disappearance of the British vineyards<sup>7</sup>. As a result, the pattern of consumption has since been largely determined by treaties, wars and tariff levels.

In 1703 the Methuen Treaty between England and Portugal allowed the import of Portuguese wine at a fixed and relatively low duty, in return for concessions on the exports of woollen cloth to Portugal<sup>11</sup>. This treaty remained in force until 1831 and

was the principal reason why the British drank port during the eighteenth century rather than claret as before. Between 1704 and 1785 the proportions of wine, by country of origin, which were consumed in Britain were Portuguese 65.4%, Spanish 29.3% and French 3.6%<sup>11</sup>. Consumption of port and other fortified wines was largely confined to the richer members of the community: for example one Dr John Campbell drank 13 bottles at one sitting and many men habitually drank 4 bottles of port a day<sup>13</sup>.

In 1783 a new treaty with France lowered the duties on French wine. This was partly designed to combat the smuggling of French wines, which was rife, and it led to an increase in consumption in the 1780s. This continued into the next century despite the Napoleonic wars and was followed by a decline in consumption in the post-war depression until 1825 when the wine duties were altered<sup>11</sup>. Consumption remained steady until 1860 when the Cobden Treaty was signed with France, and the wine duty was reduced. This was followed in 1861 by the duties charged being based on the alcoholic strength of the wine; a lower rate was levied on 'light' wines not exceeding 20° proof

Table 3 Major statutes affecting consumption

Date	Statute	Effect
1751	An Act . . . for more effectually restraining the retailing of distilled spirituous liquors	Greatly increased duty on spirits
1752	The Disorderly Houses Act	Controlled excessive numbers of public houses
1828	The Alehouses Act	Restricted opening hours
1830	The Beerhouse Act	Allowed beer sales on licensed premises only
1848	The Alehouses and Beer-houses Act	Restricted opening hours
1854	The Sunday Beer Act	Reduced opening hours on Sundays
1872	The Licensing Act	Restricted numbers of licences
1908	Children's Act	Children under 14 not allowed to enter bars
1914	The Defence of the Realm Act	Public houses closed at 11 p.m. weekdays, 10.30 p.m. Sundays
1923	An Act (on) the sale of intoxicating liquor to persons between 14 and 18 years of age	Illegal to sell intoxicating liquor to anyone under 18
1945	The Licensing Planning Act	Controlled redevelopment of public houses in bombed areas



spirit. Consumption increased during the mid-Victorian period of prosperity, but declined after 1875 because of an economic depression. Consumption of port increased despite the higher rates of duty on fortified wines, but later declined perhaps because after-dinner smoking became fashionable instead. Sherry consumption increased until 1873, when the demand was so great that poor quality wine was sold to meet this demand and led to consumer resistance<sup>11</sup>.

Apart from a small peak at 1900, consumption continued to decline up to and during World War I. During the war years wine consumption was not affected as much as beer or spirits: although imports of wine were checked and formal entertaining was curtailed, consumption was stimulated by the low-gravity beer and shortage of spirits<sup>11</sup>. Post-war celebrations doubled the consumption in 1919, but this was reversed by an increase in duty in 1920. Consumption recovered a little in the 1930s but almost no wine was available during World War II because of the shipping blockade and the fact that many of the wine-producing countries were controlled by Axis powers.

Consumption rapidly recovered after the War, encouraged by a variety of stimuli. A reduction of duty in 1973 caused a further steep increase in consumption; the increases in duty in 1976 and 1977 seem to have checked this.

### British wines and mead

Little is known about consumption of British wines before 1927 because this wine was taxed only for a few short periods. Consumption rose from 0.5 pints in 1930 to nearly 1.2 pints per person per yr in 1939, only to fall to 0.5 pints again during World War II. Only in 1960 were pre-war levels reached again. This upward trend has continued and reached 2 pints in 1975. A recent estimate shows that home wine making from kits provides 0.33 extra pints per person per yr.

### Factors affecting consumption

The consumption of alcohol depends on four factors: price, availability, current social pressures, and educational or moral campaigns<sup>11</sup>. Economic factors seem to be of paramount importance, as the fluctuations in the consumption of alcohol closely follow the rise and fall in the general trade of the country. Years of prosperity such as 1875 and 1899 were years of much drunkenness<sup>11</sup>, and this is illustrated by Booth's evidence from a London policeman during the 1880's: "a great amount of drunkenness is still a sure sign of work being plentiful. It is then that the police are busiest"<sup>15</sup>. Equally the economic depressions of 1855–60, 1875–80, 1894, 1904, 1909 and 1928–35 were years of modest consumption. Furthermore, as we have seen, consumption is sensitive to purchase price. The prices of beer, spirits and wines relative to each other, and relative to other beverages and to food and other goods in general, also affect consumption. Coffee was introduced to England in 1637 and tea and cocoa followed around 1650<sup>7</sup>. They were luxuries at first and drunk only by the wealthy, but by 1800 the price had fallen far enough to make their use universal. Tea consumption rose from 1.4 pounds per person per yr in the first decade of the nineteenth century to 5.7 pounds in the last decade (and is now 7.7 pounds). Similarly coffee consumption increased from 1.1 ounces per person per yr in 1801 to 16 ounces in 1880, but then declined. (It has recently reached about 80 ounces.) Cocoa imports grew from 0.5 ounce per person in 1822 to 10.4 ounces per person in 1894. And between 1841 and 1882 taxes on tea, sugar, coffee and corn were lowered from £15.8 million to £4.8 million, while those on beer, spirits, wine and tobacco rose from £18.1 million to £37.3 million<sup>16</sup>; this helped tea to displace beer as the main drink of the poor. The price of alcoholic beverages relative to other food has also decreased recently, and it has been estimated that the number of minutes work required from a male manual worker to pay for a large loaf (1½ pounds), 1 pint of beer and a bottle of whisky have changed from 9, 23 and 659 respectively in 1950 to 11, 12 and 209 in 1976<sup>17</sup>.

**Table 4** Decreasing contribution of alcoholic drinks to nutrient intakes (mg per person per d)

	Iron	Calcium	Magnesium	Riboflavin	Nicotinic acid
1691	0.27	150	133	0.83	15.7
1741	0.20	110	97	0.61	11.5
1791	0.14	67	59	0.37	7.0
1841	0.09	47	41	0.26	4.9
1891	0.12	60	54	0.38	6.3
1941	0.05	27	24	0.15	2.8
1971	0.10	29	25	0.10	1.5
Recommended intake*	10.0	500	300	1.7	18.0

Iron was calculated for both wines and beer, and the remaining nutrients for beer alone as any contribution from wines or spirits was insignificant. For derivation of nutrient content of earlier liquors, see text.

\*Recommended intake for a moderately active male aged 18–35yr<sup>19</sup>. The recommendation for magnesium comes from the Canadian recommended daily intakes<sup>28</sup>.

Licensing hours control consumption of all drinks on licensed premises, though any person aged 18 or over may purchase as much alcohol as he wishes to consume at home.

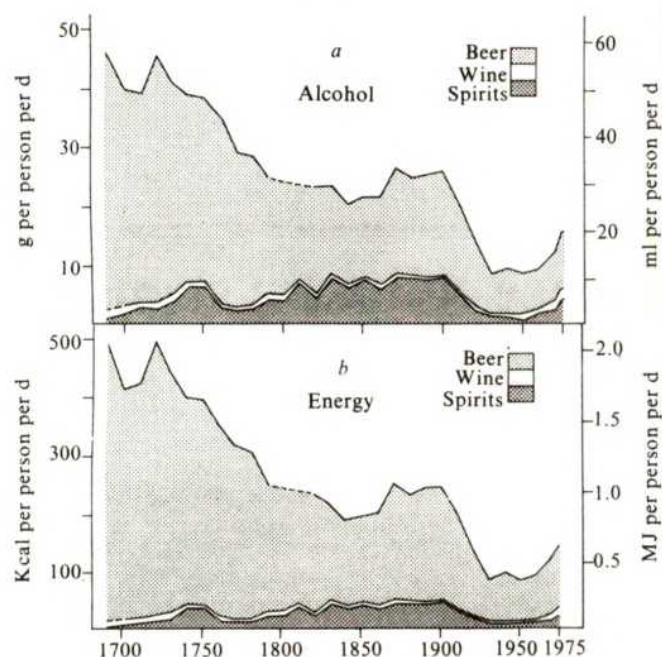
Social factors which affect consumption are of two types. Those which tend to decrease consumption include the social unacceptability of being drunk, the laws regarding drinking and driving and counter-attractions such as the availability of non-alcoholic refreshments, the acquisition of cheap consumer goods, smoking, gambling, thrift, travel, the cinema, radio and television which competed with alcohol for an individual's spending money. On the other side of the coin concern has been expressed about social conventions which lead to higher consumption in certain occupations such as business executives<sup>5</sup>; this is, however, a convention of long standing as Booth remarks on its prevalence in the 1880s<sup>15</sup>. Publicity has also been given to the so-called 'lace-curtain drinking syndrome' among those married women who can afford it; this too is not a new problem as Booth relates "All round London are growing up suburbs of small houses whose occupants have just enough to live on comfortably. Women are left at home, small ailments, the immediate stimulus of drink, that is how it begins"<sup>15</sup>. Booth also related the increase in alcohol consumption in women in the 1880s to their emancipation and increasing financial independence from men. In recent years the sale of alcohol in supermarkets, and liquor trade advertisements equating alcohol with glamorous living, may have tended to increase consumption.

The temperance movement has been the most prominent moral and educational force to affect consumption. The Victorian middle classes were horrified by drunkenness and especially by its increase amongst the working classes as they became more prosperous. Although their ancestors had accepted excessive consumption without question, they began to denounce it as an unmitigated evil. Previous social reformers had denounced spirits but had recognised beer as a cheap and wholesome drink greatly preferable to impure water, but from the 1850s temperance reformers zealously condemned all alcoholic beverages and achieved much in the imposition of restrictions on the sale of drink to children (see Table 3). The temperance movement was a formidable demonstration of the power of organised middle-class opinion and of the 'non-conformist conscience'<sup>18</sup>.

### Contributions to diet

The relative contributions made by beer, spirits and wines to the diet are disguised when each is recorded in terms of volume. Spirits have a very high energy content and 1 proof pint (equivalent to about 1.42 pints at 70° proof) provides about 7.5 MJ or 1,800 kcal, but essentially no other nutrient. Wines contain much less alcohol, even when fortified, and despite their





**Fig. 2** Relative contributions of beer, spirits and wine to the diet; *a*, in terms of alcohol and *b*, in terms of energy. Values for 1821, 1831, 1951, 1961, 1971 and 1975 include the contributions made by cider and British wines. The alcohol and sugar contents of beer at original gravity 1,055° were each taken as 4.2 g per 100 ml (ref. 10); the alcohol content of spirits is fixed when quantities are recorded in proof gallons; and wines were calculated on the assumption that French, Rhenish and Empire or Commonwealth wines were 'light' and could be treated as an equal mixture of red, rose and white wines, while wines from Portugal, Spain and their colonies were 'heavy' and could be treated as an equal mixture of port, sherries and vermouth. The alcohol and energy values of these and cider were then derived from standard tables of food composition<sup>20</sup>. Any errors in the assumptions necessary for wines are trivial while consumption has been so low.

sugar content they also provide less energy. The small amounts of iron and other nutrients are of little significance when the low consumption is averaged over the whole population. Beer contains the least alcohol, but in the amounts drunk its contribution to the energy (with 1 pint as drunk now providing between 0.6 and 1.7 MJ) and B-vitamin content of the diet can be substantial.

Figure 2 shows the amounts of alcohol and of energy provided by beer, wines and spirits. Although they are expressed per head of the population, each has been restricted to certain sections of the population, each of which would have drunk much more than the averages illustrated. Even so, it is obvious that the total amount of alcohol available has declined fairly steadily. Beer has always provided the most alcohol although the percentage has in general declined and now stands at 67%. Spirits have never provided more than a third of the total alcohol available; they now provide about 20%. Wine has recently become relatively more important.

The energy provided has changed almost exactly in parallel with the alcohol content (Fig. 2). The main difference is that spirits, which contain little or no carbohydrate, make a relatively smaller contribution to energy than to alcohol. The three beverages together provided between 1.5 and 2.5 MJ (400–500 kcal) per person per d in the first half of the eighteenth century, but this had fallen to less than 1 MJ (200 kcal) 100 yr later. After a rise to just over 1 MJ (250 kcal) in the latter part of the nineteenth century, it remained at an historical low of about 0.4 MJ (100 kcal) from 1930 to 1960 but has now increased again to about 0.6 MJ (150 kcal) per person per d.

Beer is and always has been the greatest source of energy as alcohol (on average) although its contribution has fallen from 92% of the total from all alcoholic drinks in 1731 to 77% in

1831, and from 84% in 1931 to 79% in 1971. Spirits have provided up to one-fifth of the total, while the contribution from wine, although small, has increased from 5% in 1731 (when most of the wine was fortified) to 9% in 1971 despite the relatively recent increase in the proportion of light wines with only two-thirds the energy value of their fortified counterparts.

It is not possible to calculate the relative importance of food and alcohol to the diet for most of the period covered, because few national surveys of the total food supply were attempted before 1940. We have, however, estimated the average energy requirement of the population at selected times, to determine what proportion of this was likely to have been satisfied by alcoholic drinks. The different age and sex structures of the population (particularly the greater proportion of children in earlier years) were taken into account from Census data, and present recommendations<sup>19</sup> for each group were then applied to these on the assumption that in the nineteenth century all men and women were 'very active' and in the twentieth century they were 'moderately active'. The average requirement of the population was then approximately 10.3 MJ (2,460 kcal) per person per d in 1821, 10.5 MJ (2,500 kcal) in 1871 and 10.8 MJ (2,570 kcal) in 1911 compared with about 9.8 MJ (2,350 kcal) in 1971; the proportions met by the alcoholic drinks available thus averaged 10% in 1821 and 1871 and 8% in 1911, compared with about 5% in 1971.

Table 4 shows the amounts of three minerals (iron, calcium and magnesium) and two B vitamins (riboflavin and nicotinic acid) estimated to have been provided by the beer available in selected earlier years. The minor contributions of the different wines to iron intakes are also included, but the amounts of other nutrients provided by alcoholic drinks were insignificant.

Values for 1971 were derived from standard tables of food composition<sup>20</sup>. Earlier values are the best estimates we can make in the virtual absence of direct information, and were obtained as follows. The B vitamins in beer, being derived mainly from the malt, seem to be related to the strength of the brew; thus, a sample of strong ale supplied to Queen's College, Oxford before World War II contained 0.39 mg riboflavin per 100 ml (ref. 2) compared with 0.06 mg in strong ale and 0.04 mg in draught bitter brewed recently<sup>20</sup>. Other analyses of beer in the 1930s and 1940s showed more riboflavin and nicotinic acid than in modern beers—0.08 mg per 100 ml (including one bottle brewed in 1798)<sup>21</sup> and 1.5 mg per 100 ml (ref. 22) respectively. These values were used in assessing intakes for 1941 and earlier. The vitamin and mineral contents of beers in previous centuries have thus been related to their original gravity.

Given these assumptions beer (and wine) seems to have made a major contribution to the nutritional value of diets in the seventeenth and eighteenth centuries, and especially to riboflavin, nicotinic acid, calcium and magnesium intakes.

We thank the librarians of the Customs and Excise Department for their help in making so many old records available, and the Trade Associations and many brewers and other individuals who provided information and advice.

1. *Report on Drinking and Driving* (Department of the Environment, HMSO, London, 1976).
2. *First Report from the Expenditure Committee* (House of Commons, HMSO, London, 1977).
3. *The Brewers Statistical Handbook* (Brewing Publications, London, 1975).
4. *The Registrar General's Statistical Review of England and Wales 1963–1973* (HMSO, London, 1973).
5. *Report of the Working Party on Alcohol and Work* (National Council on Alcoholism, London, 1976).
6. Ministry of Agriculture, Fisheries and Food, *Trade and Industry* 12, 459–466 (1973); 29, 366–368 (1977).
7. Wilson, C. A. *Food and Drink in Britain* (Constable, London, 1973).
8. Parreaux, A. *Daily Life in England in the Reign of George III* (Allen and Unwin, London, 1966).
9. Williams, E. N. *Life in Georgian England* (Batsford, London, 1962).
10. *Foods Standards Committee Report on Beer* (Ministry of Agriculture, Fisheries and Food, HMSO, London, 1977).
11. Wilson, G. B. *Alcohol and the Nation* (Nicholson and Watson, London, 1940).
12. Clutton, D. W. *Flav. Ind.* 3, 454–456 (1972).
13. Burton, E. *The Georgians at Home* (Arrow, London, 1973).
14. *The Fifth Report of the Commissioners Enquiring into the Collection and Management of Public Revenue in Ireland and Certain Departments in Great Britain* (HMSO, London, 1862).



15. *Charles Booth's London* (eds Fried, A. & Elman, R.) (Penguin, London, 1971).
16. Burnett, J. *Plenty and Want* (Pelican, London, 1966).
17. *Hansard* 927, 61 (1977).
18. Reader, W. J. *Life in Victorian England* (Batsford, London, 1974).
19. *Reports on Public Health and Medical Subjects* No. 120 (Department of Health and Social Security, HMSO, London, 1969).
20. McCance, R. A. & Widdowson, E. M. in *The Composition of Foods* 4th edn. (eds Paul, A. A. & Southgate, D. A. T.) (HMSO, London, 1978).
21. Hopkins, R. H. *Nature* 152, 274 (1943).
22. Drummond, J. & Moran, T. *Nature* 153, 99–100 (1944).
23. Monckton, H. A. *A History of English Ale and Beer* (Bodley Head, London, 1966).

24. *Annual Reports of HM Customs and Excise Departments* (HMSO, London).
25. *The First Report of the Commissioners of the Inland Revenue* (HMSO, London, 1857).
26. *First Abstract of Historical Statistics* (eds Mitchell, B. R. & Deane, P.) (Cambridge University, Cambridge, 1962).
27. *The British Economy Key Statistics 1900–1970* (Times Newspapers, London, 1971).
28. *Dietary Standard for Canada* (Department of National Health and Welfare, Supply and Services, Ottawa, 1975).

# Interparticle forces in multiphase colloid systems: the resurrection of coagulated *sauce béarnaise*

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*The successful preparation of sauce béarnaise is reported. The physico-chemical factors influencing the stability of this colloidal system are considered.*

VARIOUS authors have extensively described<sup>1–5</sup> the preparation of *sauce béarnaise* (SB), the colloquial name for a (hopefully) stable colloidal suspension consisting of a hydrophobic phase suspended in a low pH aqueous phase at different ionic strengths. It is highly probable that the colloidal particles are micelles consisting of a mixture of phospholipids, fats, proteins, cholesterol and various long-chain unsaturated fatty acids. The aqueous phase contains mainly acetic acid and sodium chloride at ionic strengths determined by the initial conditions. Chlorophyll-containing additives, we believe, have little influence on the colloidal properties of the system.

We shall first give a brief description of the underlying physico-chemical principles. Hydrophobic colloidal suspensions exist because of an interplay of double-layer repulsion and attractive dispersion forces. The latter are relatively independent of the composition of the dispersive medium and temperature although in some circumstances this assumption cannot be justified<sup>6–9</sup>. The double-layer repulsions arise from the surface charge resulting from ionisation of adsorbed acetic acid, in this case, screened by the dispersing electrolyte solution<sup>10</sup>. This contribution to the forces can clearly be influenced by the conditions prevailing in the dispersive medium. The aim of this article is to indicate how an understanding of this interplay of forces can lead to more complete experimental control of the stability of this extremely complex and important system.

## Theory

Although we can find no definitive light or neutron scattering data on this system, we assume that the micelles are spherical, and hence that the Van der Waals forces between them are well described by the equation of Hamaker<sup>11</sup>

$$U_A = -\frac{A}{6} \left[ \frac{2}{a^2 - 4} + \frac{2}{a^2} + \ln \frac{a^2 - 4}{a^2} \right] \quad (1)$$

where  $U$  is the interaction energy relative to infinite separation,  $a$  is the non-dimensional interparticle separation scaled with respect to the particle radius and  $A$  is the so-called Hamaker constant, with a value of about  $10^{-12}$  erg. The number of adsorbed acetic acid molecules per unit area of the particles may be predicted from the ambient bulk concentration and a suitable adsorption isotherm<sup>12,13</sup>. The fraction of these which are dissociated will naturally depend on the composition of the dispersive medium,

particularly the pH. Furthermore, we can expect that the solvation by acetic acid of the ionic groups on the colloid particles will significantly affect the stability of the particles<sup>14</sup>. If there are  $N_s$  dissociated acid groups per unit area these contribute a surface charge

$$\sigma_N = N_s q \quad (2)$$

where  $q$  is the protonic charge. The ionic strength

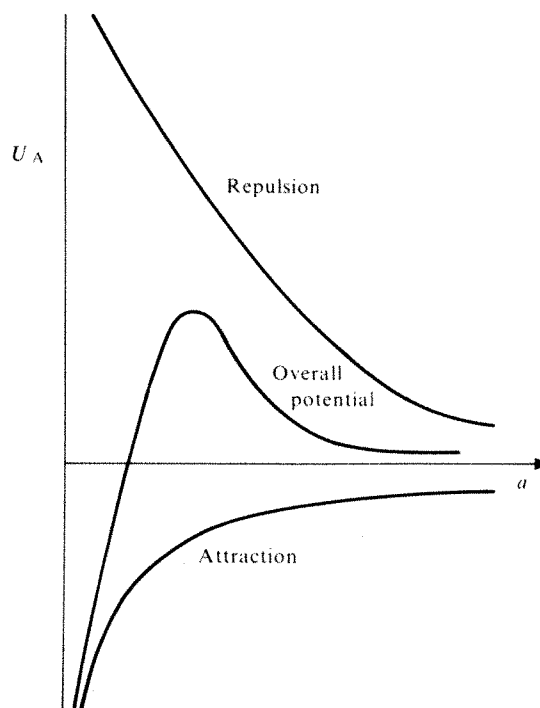
$$\mu = \frac{1}{2} \sum_{i=1}^N \rho_i z_i^2 \quad (3)$$

of the dispersing medium will result in a screening of the coulombic repulsion due to the negative charges on the particles' surfaces. The potential of these forces decays with distance  $r$  according to an exponential law<sup>6</sup>:

$$F \sim \psi_0^2 [1 - \tan h \kappa L/2]$$

where:  $\psi_0$  = surface potential,  $\kappa$  = inverse Debye length, and

Fig. 1 Potential curves for the interparticle interaction in a multiphase colloid system.



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**Table 1** Consistency of *sauce béarnaise*

Observer no.	No. of samples	Completely homogeneous	Partially homogeneous	Heterogeneous
1	2	2	—	—
2	2	2	—	—
3	2	2	—	—
4	3	3	—	—
5	2	2	—	—
6	2	2	—	—
7*	2	2	—	—
8*	3	3	—	—
9*	3	3	—	—

\*The authors.

 $L$  = distance between the particle surfaces.

The various contributions and overall potential energy curve are shown in Fig. 1. If the height of the maximum in the potential curve is large compared to  $kT$ , where  $k$  is Boltzmann's constant and  $T$  the absolute temperature, then the sauce (SB) will be stable.

This maximum will be increased by the surface charge and reduced by increasing either temperature or ionic strength. This accounts for the empirical observation that the application of multivalent ions has never been recommended in the manufacture of *sauce béarnaise*. Only the increase of  $\sigma$  can have a beneficial influence on the stability of the product.

### Experimental methods

Commercially available reagents (vinegar, onion, egg yolks, butter, parsley, tarragon, *herbes de Provence*, mustard, pepper, NaCl and alpine water ( $H_2O$ )) were used, according to ref. 3. We do not expect that our conclusions would have been altered if the alternative procedures<sup>1,2,4,5</sup> had been used. In our hands, heavy

coagulation was observed despite vigorous stirring and careful temperature control ( $\pm 5$  K). When coagulation occurs, other authors recommended discontinuation of the experiment<sup>1-5</sup>. However, based on the above theoretical considerations, we decided to add, with extremely vigorous stirring, a further quantity of the commercially available acetic acid solution, and the results confirmed our theoretical predictions.

### Results and discussion

After addition of acetic acid, and as vigorous stirring proceeded, the heterogeneous phase soon assumed the expected homogeneous consistency. Examination of the resulting preparation was immediately undertaken by a significant number of trained observers. The results of this examination are listed in Table 1. To our knowledge, this is the first successful attempt to resurrect a *sauce béarnaise* based on the theory of the stability of lyophobic colloid<sup>10</sup>.

Thanks are due to Drs K. Hildenbrand, A. Reimann and to M. Hildenbrand, V. Hallmann, H. Dittich and I. Hengst for agreeing to take part in the experiment.

1. Brillat-Savarin, A. *La physiologie du goût* 115 (Flammarion, Paris, 1929).
2. Wilmenrod, C. *Französische Küche* 65 (Vollmer, Wiesbaden-Berlin, 1973).
3. Bertholle, L. *Die geheimen Rezepte der besten Restaurants Frankreichs*, 489 (Hallwag Bern, Stuttgart, 1976).
4. Larousse (ed.) *Larousse Gastronomique* (Paris, 1962).
5. Banzer-Friebel in *Die Hotel- und Restaurationsküche* (ed. Friebel, C.) 84 (Fachbuchverlag Dr Planneberg, Giessen, 1974).
6. Barouch, E., Perram, J. W. & Smith, E. R. *Chem. Phys. Lett.* **19**, 131-133 (1973).
7. Barouch, E., Perram, J. W. & Smith, E. R. *Stud. appl. Mathematics* **11**, 175-186 (1973).
8. Barouch, E., Perram, J. W. & Smith, E. R. *Proc. R. Soc. A* **334**, 49-55 (1973).
9. Barouch, E., Perram, J. W. & Smith, E. R. *Proc. R. Soc. A* **334**, 59-71 (1973).
10. Verwey, E. J. W. & Overbeek, J. T. G. *Theory of the Stability of Lyophobic Colloids* 139 (Elsevier, Amsterdam, 1948).
11. Hamaker, H. C. *Physica* **4**, 1058-1067 (1931).
12. Langmuir, I. *J. Am. Chem. Soc.* **38**, 2221 (1916).
13. Perram, J. W. & Smith, E. R. *Proc. R. Soc. A* (in the press).
14. Nicolau, C., Dreeskamp, H. & Schulte-Frohlinde, D. *FEBS Lett.* **43**, 148-150 (1974).

# articles

## New data on climatic trends

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*Indicators of large-scale climate developments show that the oscillatory cooling observed in the past 30 yr in the Northern Hemisphere has not yet reversed. This conclusion was reached by updating our data on the month-to-month, season-to-season, and year-to-year variations of selected zonally averaged meteorological parameters.*

THE reported set of climatic indices shown in Figs 1 and 2 represent large segments of the Earth-atmosphere system in both hemispheres. Changes in the average surface air temperatures in

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the Northern Hemisphere from 1951 to 1975 are shown in Figs 3-5 by curves 101-111, prepared by R. Yamamoto *et al.*<sup>1,2</sup>. Curves 101-105 show the relative departures of the 12 month running means for designated latitudinal zones. Curves 106 through 111 in Figs 4 and 5 show the departures for spring (March-May), and autumn (September-November) respectively. Data from 358 stations, north of 20°S (see Fig. 1) were analysed. They were obtained from the *World Weather Records*<sup>3</sup> and *Monthly Climatic Data for the World*<sup>4</sup> and calculated as departures from the 1951-75 mean. An interpolating method of cubic spline under tension, developed by Cline<sup>5</sup>, was used in the

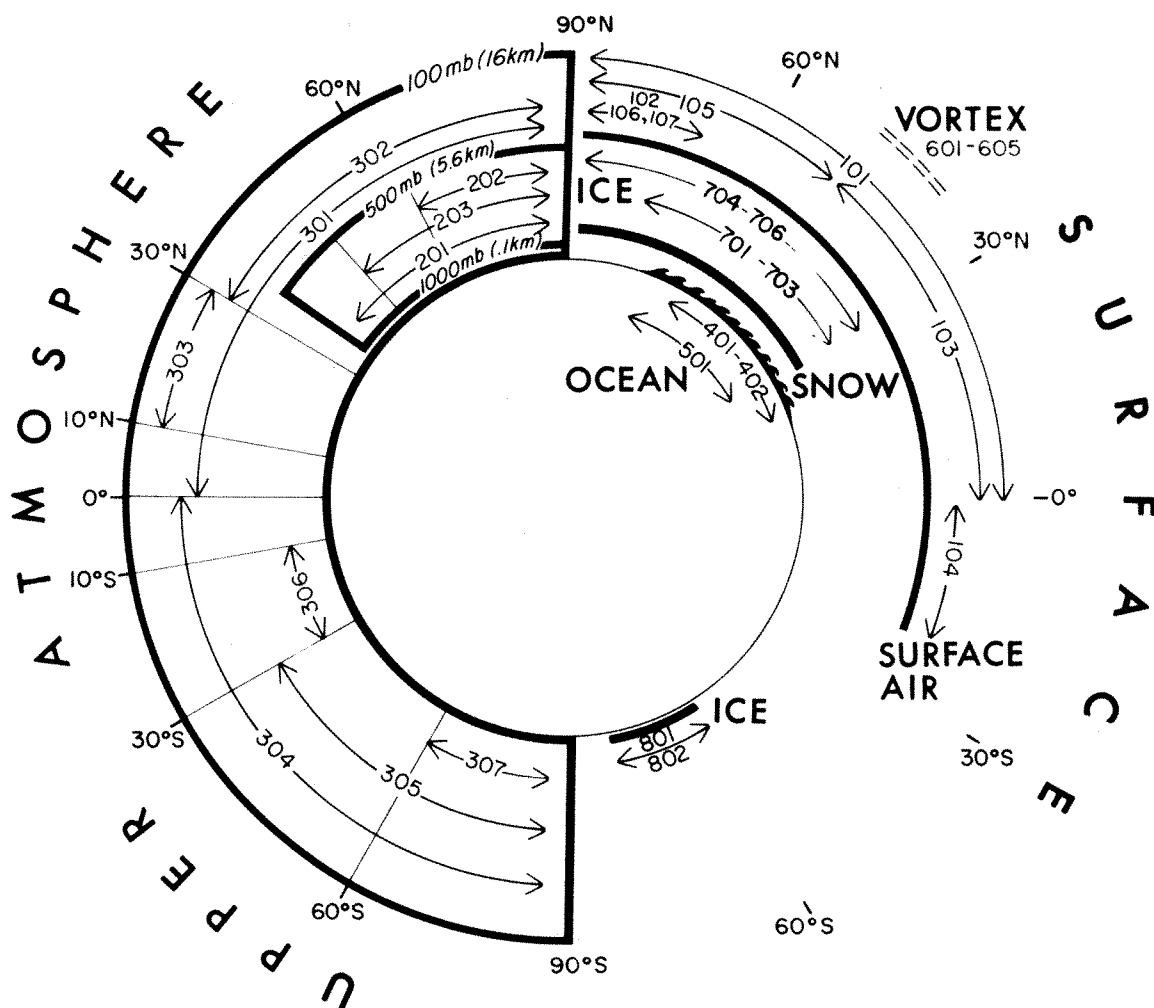


Fig. 1 Area represented by climatic indices listed in Table 1

analysis. The details of the averaging technique are given elsewhere<sup>1,2,5</sup>. Frequently climate is defined by parameters measured 1.5–2 m above the ground, where most of the meteorological instruments are situated. Because the surface air temperatures correspond directly to this level, curves 101–111 are the most direct indicators of surface climate fluctuations included in our set. But, the large geographic variability in surface temperature trends and uneven distribution of observing stations severely limits the accuracy of our estimates. The results should be most reliable for the middle and high latitudes of the Northern Hemisphere where the station density is highest. The striking feature of the record is the large range of year-to-year temperature fluctuations. A progressive temperature drop over the recorded interval with oscillations about 3–4 yr long and an intensive cooling episode between 1961 and 1964, can be seen. The data are in general agreement with earlier work<sup>6–11,46–49</sup>. It is interesting that during the past decade the high latitudes of the Northern Hemisphere show warming in the spring (Fig. 4, curve 106) but cooling in autumn (Fig. 5, curve 107). Only a slight change in the spring and autumn temperature is seen south of 50°N.

### Free atmosphere below 500 mbar

The relative temperature of the free atmosphere between the 500-mbar and 1,000-mbar levels in the middle and high latitudes of the Northern Hemisphere from 1949 to 1976 was studied. The annual average departures from the 1949–73 mean are plotted in curves 201–203 in Fig. 3. The data were obtained by H. Dronia<sup>12,13</sup> through the analysis of monthly pressure charts<sup>14</sup>. Approximately the lower 5.5 km of the troposphere is described. Information on the heights of the 500-mbar and 1,000-mbar levels was read at 220 gridpoints at intervals of 5° latitude and 10° longitude,

which cover about 55% of the hemisphere (Fig. 2). The average relative temperature was calculated from the changing thickness of the atmospheric column between the two limiting pressure levels. A decrease in thickness of 20 m corresponds to an average temperature drop of 1 °C. A detailed discussion of the method is given elsewhere<sup>12,13</sup>. The data are more spatially homogeneous than ground level temperatures. It is possible that the upper atmosphere data include a small, as yet unknown 'instrumental' contribution to the cooling trend caused by gradually improved USSR radiosondes. This would lessen the average rate of the cooling trend as observed since the 1950s. Further studies seem necessary<sup>34</sup>.

Perhaps the most striking feature of the record is the large year-to-year variability. The curves show a gradual oscillatory cooling through the studied interval. A record negative departure in the mid-latitudes (Fig. 3, curve 201) accompanied by relative warming in high latitudes (curve 202) occurred in 1976. Rapid cooling of the atmosphere between 1959 and 1964, first reported by Starr and Oort<sup>15</sup> is also evident.

### Free atmosphere below 100 mbar

The relative temperature of the free atmosphere between the 100-mbar level and surface from 1958 to 1976 was analysed by Angell and Korshover<sup>16,17</sup>. The data are shown separately for different latitudinal belts of both hemispheres by curves 301–307. The Southern Hemisphere south of 30°S is represented by only 12 stations so that the results are highly tentative. The fluctuations in atmospheric temperatures north of 30°N are probably more accurately indicated by Dronia's curves 201–203, despite the fact that these curves refer only to the column below 500 mbar. But

Table 1 List of plotted indices

Curve	Parameter	Area	Units	~ Height	Interval	Average	Authors	Figure
101	SAT*	0–90°N	0.2 °C	—	1951–75	12-month run	Yamamoto Iwoshima Hoshiai	3a
102	"	70–90°N	"	—	"	"		3a,b
103	"	0–50°N	"	—	"	"		3a
104	"	0–20°S	"	—	"	"		3a
105	"	50–90°N	"	—	1965–75	"		3b
106	"	70–90°N	"	—	"	Spring		4
107	"	70–90°N	"	—	"	Fall		5
108	"	50–90°N	"	—	"	Spring		4
109	"	50–90°N	"	—	"	Fall		5
110	"	0–50°N	"	—	"	Spring		4
111	"	0–50°N	"	—	"	Fall		5
201	ATM†	35–90°N	0.2 °C	1,000–500 mbar (0.1–5.6 km)	1949–76	Annual	Dronia	3a
202	"	65–90°N	"	"	"	"		3a,b
203	"	50–90°N	"	"	1965–76	"		3b
301	ATM	0–90°N	0.2 °C	Surface–100 mbar (0–16.0 km)	1958–76	Annual	Angell Korshover	3a
302	"	30–90°N	"	"	"	"		3a
303	"	10–30°N	"	"	"	"		3a
304	"	0–90°S	"	"	1958–75	"		3a
305	"	30–90°S	"	"	"	"		3a
306	"	10–30°S	"	"	"	"		3a
307	"	60–90°S	"	"	"	"		3a
401	SST‡	N. Central Pacific	0.2 °C	—	1947–77	12-month run	Namias	3a
402	"	"	"	"	"	Monthly		6
501	SST	N. Atlantic Stations (NOAS)	0.2 °C	—	1951–72	5-yr run	Rodewald	3a
502	"	Central N. Atlantic (Station C)	"	—	1966–76	"		3a
503	"	"	"	—	1967–76	12-month run		3b
504	"	Boothbay Harbor	"	—	1965–76	Annual		3b
601	VOR§	N. Hem.	Percent	300 mbar (9.2 km)	1966–76	12-month run		3b
602	"	"	"	"	"	Spring	Angell Korshover	4
603	"	"	"	"	"	Fall		5
604	"	"	"	"	"	Summer		4
605	"	"	"	"	"	Winter		5
701	SNO¶	N. Hem.	Millions km <sup>2</sup>	—	1966–77	12-month run	Matson Wiesnet	3b
702	"	N. America	"	—	"	"		3b
703	"	Europe Asia	"	—	"	"		3b
704	SIC	N. Hem.	Millions km <sup>2</sup>	—	1967–77	12-month run	Kukla	3b
705	"	"	"	—	1967–76	Spring		4
706	"	"	"	—	"	Fall		5
801	ICE**	S. Hem.	Millions km <sup>2</sup>	—	1973–77	12-month run		3a
802	"	"	"	—	"	End of Nov.		3a

Temperature departures:

\*SAT, Surface air.

†ATM, upper atmosphere.

‡SST, Sea surface.

Area changes:

§VOR, relative vortex area (%).

¶SNO, Snow on land.

||SIC, Snow and pack ice.

\*\*ICE, Pack ice.

the latter are based on data from a larger number of stations. The average annual heights and temperatures of the analysed pressure levels were computed from the *Monthly Climatic Data for the World*<sup>4</sup>. The changes of average thickness were converted to mean annual temperature departures based on assumptions similar to those made by Dronia<sup>12</sup>. Details of the procedure are described elsewhere<sup>16</sup>.

Apart from the large year-to-year changes, a gradual oscillatory drop of atmospheric temperatures is observed in all curves except the 60–90°S, which shows warming. Data for zones poleward of 30°N are in general agreement with Dronia's curves 201–203 (Fig. 3). Large oscillations but no pronounced trend can be seen in the low latitudes of both hemispheres between 30°N and 30°S. Angell and Korshover<sup>17</sup> reported essentially no change in the temperature of the global atmospheric column (90°N–90°S) averaged with respect to mass between 1971 and 1976.

### Pacific sea-surface temperatures

Sea-surface temperatures (SST) in the North Central Pacific are shown for 1947–77. The data were analysed by Namias<sup>18</sup> as

departures from the 1947–66 mean. They are shown in curves 401 (Fig. 3) and 402 (Fig. 6). The time series is based on ship observations received by the National Marine Fisheries Service of NOAA. Over 20,000 ship injection temperatures for each month were averaged separately for 2° × 2° Marsden squares, analysed, and then smoothed on to a 5° × 5° grid centred at the intersection point. Data for the resulting 153 oceanic grid points were weighted by the relative area at each latitude and averaged. Details of the data collection and averaging are described by Namias<sup>18,19</sup>.

The large month-to-month and year-to-year variability is the outstanding feature of the record. The progressive oscillatory drop of average temperatures from approximately 1959, the increased amplitude of fluctuations from 1963 onwards (Fig. 6), pronounced cooling between 1963 and 1964, and 1968 to 1972, marked warming between 1965 and 1967, and the extreme departure of August 1976 temperatures from normal are also apparent. The largest negative departures frequently occurred in the warmest season between July and October (Fig. 6).



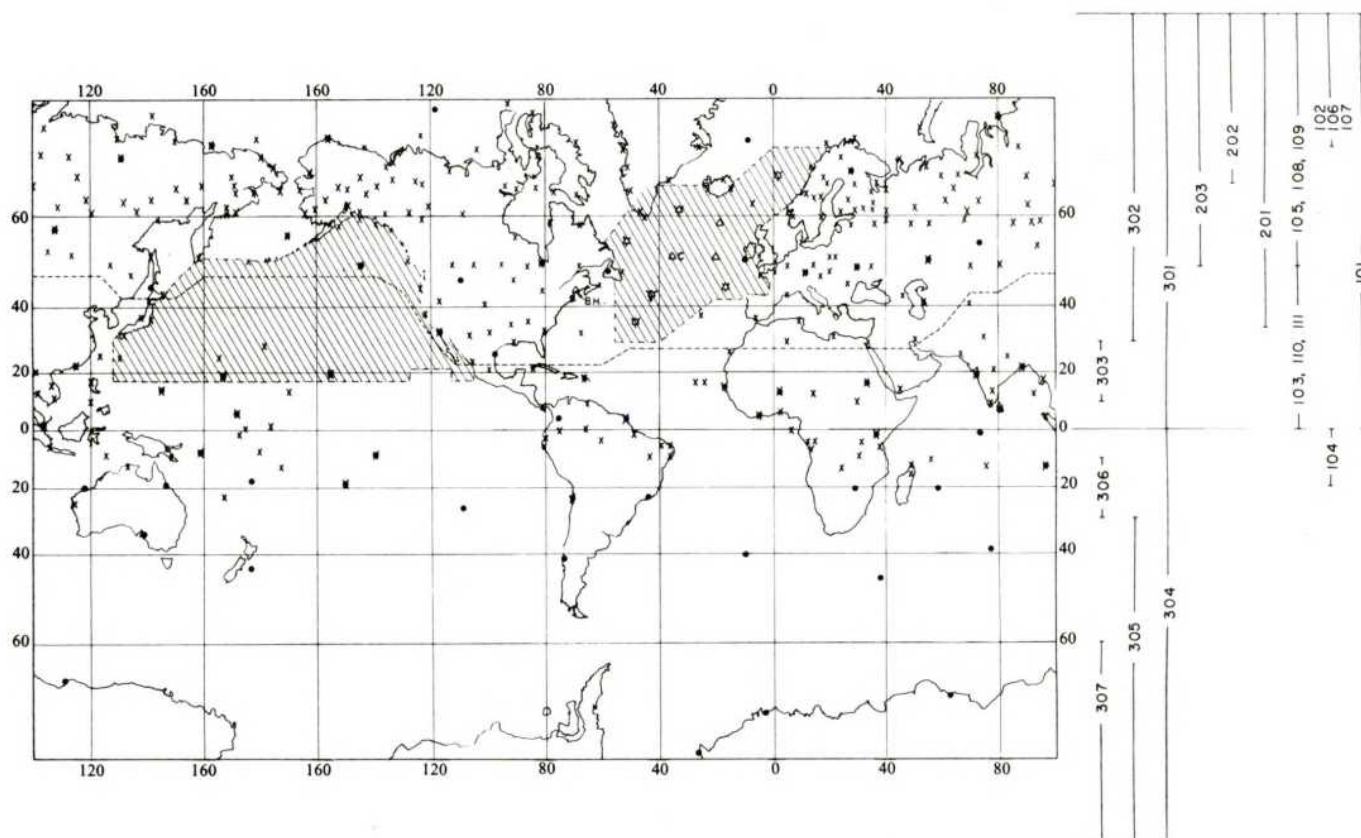


Fig. 2 Station network (polewards of 75° not shown). x, Air surface temperature (series 100); ●, upper atmosphere temperatures (series 300); Δ, ship stations; BH, coastal station Boothbay Harbour. Hatched areas, sea surface represented by curves 401, 402, and 501; dashed line, southern border of the area represented by curve 201.

The secular change in sea-surface temperature is not evenly distributed<sup>41</sup>. During the winter of 1977, for example, a cold anomaly of  $-2^{\circ}\text{C}$  was centred around  $180^{\circ}\text{W}$  and  $40^{\circ}\text{N}$ , and also affected coastal waters off Alaska and continental United States. At the same time in the vicinity of  $130^{\circ}\text{W}$  and  $40^{\circ}\text{N}$  the water was warmer than the long-term mean by  $1.5^{\circ}\text{C}$ . The records available from United States coastal stations<sup>20</sup> show a relatively warm ocean in 1957 and 1958 but generally cold from 1971 onwards. The widespread cold anomalies of coastal SST occurred in 1933–34 and 1943–44 but these were not as intense and long lasting as in the 1970s.

### Atlantic sea-surface temperatures

Sea-surface temperatures in the North Atlantic from 1951–1976 were analysed by Rodewald<sup>21</sup> who averaged the information collected from nine North Atlantic Stations (NOAS) (Fig. 1). The results are shown by curve 501, in Fig. 3a as 5-yr running means. After withdrawal of US weather ships in 1973, only two of the original nine observing stations remained occupied, C and M. Comparison of station C's record before 1973 with that of the NOAS stations shows a parallel slope in the pentadal running means (curve 502, Fig. 3a). Station C is, therefore, considered an example, to some extent, of the general trend in the North Atlantic after 1973 (curve 503, Fig. 3b). Curve 504 from Boothbay Harbor, Maine, represents the changes observed along the northeastern seaboard of North America<sup>22</sup>. Curves 501 and 502 combined, indicate a progressive drop in average SSTs, which from 1951 through 1976 amounted to approximately  $0.75^{\circ}\text{C}$ . The drop was interrupted by a temporary warming between 1963 and 1966. The largest cold departures for station C (curve 503) were observed in summer, between June and September.

### Polar vortex extent

The area of the 300-mbar northern circumpolar vortex between 1966 and 1976 was measured by Angell and Korshover<sup>23,24</sup>. The area north of the main belt of westerlies at  $40^{\circ}$ – $50^{\circ}\text{N}$  latitudes was

identified and planimeted. The contours were taken as 9,280 m for spring and autumn, 9,120 m for winter, and 9,440 m for summer, as determined from the mean monthly polar stereographic maps issued by the Free University of Berlin.

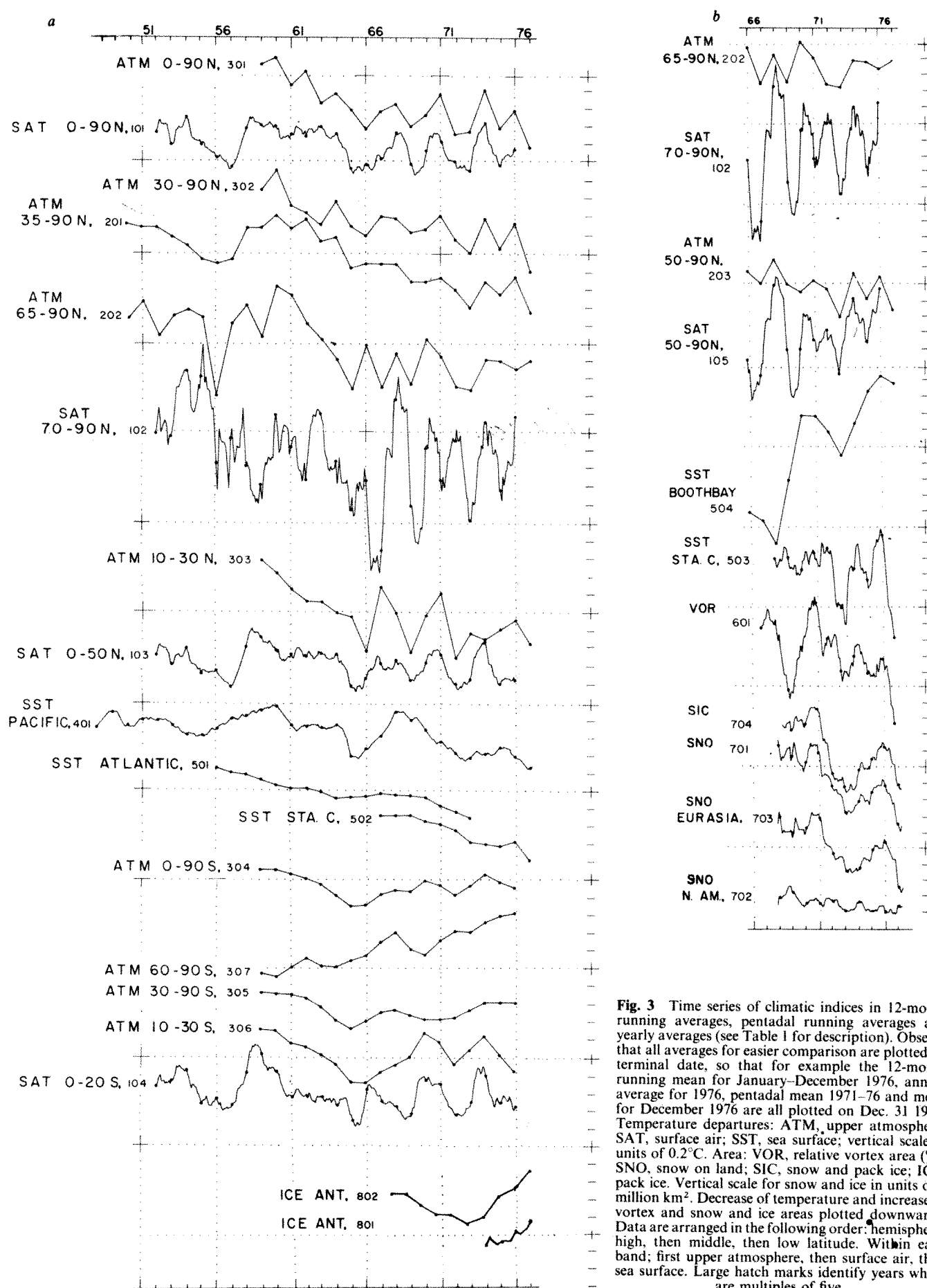
Figures 4 and 5 show that in the spring, summer, autumn and winter of 1976 the vortex area was the largest for the 10 years of record. The vortex area for the 1976–77 winter was 4% larger than the average for previous winters and more than 1% larger than any previous winter observed. The total area for 1976 was well above average and was significantly larger than the previous peak in 1968.

Quiroz<sup>25</sup> studied the central height of the polar vortex at the 10-mbar level for the winter seasons of 1966–76. He found the values to be relatively high (close to 29 km) in the winters of 1968–69, 1969–70, and 1972–73, but low (close to 28 km) in 1966–67, 1971–72 and particularly low in 1975–76. Data for the winter of 1976–77 have not been reported yet.

### Northern Hemisphere snow and ice

The seasonal and year-to-year variation of the area covered by snow in the northern hemisphere is shown in Figs 3b–5, by curves 701–706 with increasing area plotted downwards. The data are an update of measurements published earlier<sup>26–30</sup>. The area is expressed in millions of  $\text{km}^2$  and includes snow and pack ice of three classes of relative reflectivity as delimited in the NOAA weekly charts<sup>30</sup>. The maps are prepared from a visual interpretation of 6 d of satellite imagery in visible light and infrared. Details are given in refs 26–29. Interpretation of satellite imagery is not without problems<sup>50</sup>.

The large year-to-year variability, considerably more pronounced in Eurasia (curve 703) than in North America (curve 702), is the striking feature of the record. There were only minor changes in the North American winter extents. The greatest variance was observed in autumn (Fig. 5) and in Asia (Fig. 3b). It can be also seen that the autumn extent of snow and ice gradually increased between 1967 and 1972 and decreased between 1973 and



**Fig. 3** Time series of climatic indices in 12-month running averages, pentadal running averages and yearly averages (see Table 1 for description). Observe that all averages for easier comparison are plotted on terminal date, so that for example the 12-month running mean for January–December 1976, annual average for 1976, pentadal mean 1971–76 and mean for December 1976 are all plotted on Dec. 31 1976. Temperature departures: ATM, upper atmosphere; SAT, surface air; SST, sea surface; vertical scale in units of 0.2°C. Area: VOR, relative vortex area (%); SNO, snow on land; SIC, snow and pack ice; ICE, pack ice. Vertical scale for snow and ice in units of 1 million km<sup>2</sup>. Decrease of temperature and increase of vortex and snow and ice areas plotted downwards. Data are arranged in the following order: hemisphere; high, then middle, then low latitude. Within each band; first upper atmosphere, then surface air, then sea surface. Large hatch marks identify years which are multiples of five.

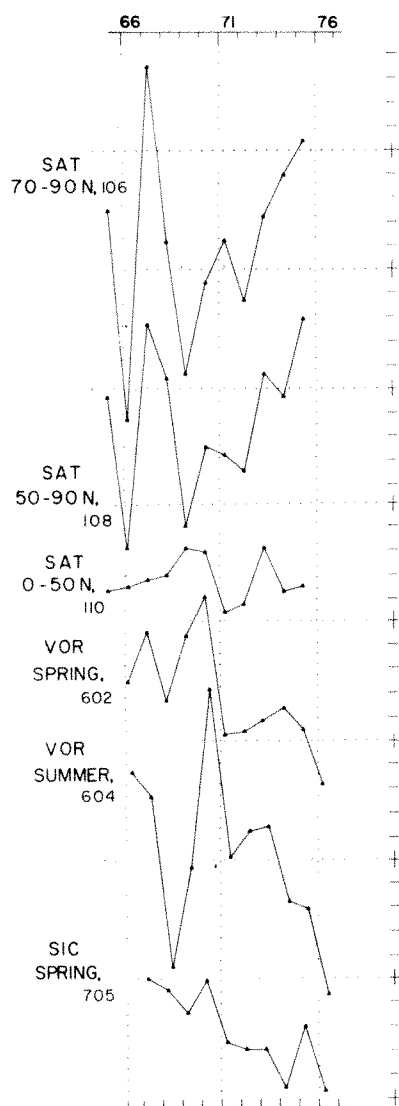


Fig. 4 Average climatic indices for spring (March–May) and vortex area for summer (June–August). Symbols and scale as in Fig. 3.

1975 (Fig. 5). The spring totals, however, increased throughout most of the interval from 1966 to 1975. Spring snow has a large impact on the surface heat balance of the continents because it reflects a significant part of incoming radiation which at this time of the year is high. Thus, the increased spring snow cover of the few past years has meant, in general, a decrease in the amount of solar radiation absorbed by the Earth's surface.

While our satellite-derived data show snow and pack-ice area combined, Sanderson<sup>31</sup> measured the sea-ice extent alone in different sections of the Arctic between 1966 and 1974 using the *Monthly British Ice Charts*<sup>32</sup>. The data indicate a decrease in the winter and spring pack-ice area in most sectors after 1969. This decrease is especially pronounced in February and April. Haupt and Kant<sup>33</sup> made similar observations on pack ice in the Barents Sea. There the maximum spring coverage occurred in 1960 and the minimum in 1973. Thus the heavy snow season of 1972–73 was associated with a relatively open Arctic Ocean and in particular with a relatively ice-free Barents Sea.

### Antarctic pack ice

The pack-ice area is shown by curves 801 and 802, in Fig. 3a (increasing area downwards). The data were analysed by Kukla<sup>27</sup>. Information on sea ice in the Antarctic waters was obtained from charts routinely produced in weekly intervals by the Fleet Weather Facility (FLEWEAFAC) of the US Navy, based on interpretation of satellite images obtained from NOAA,

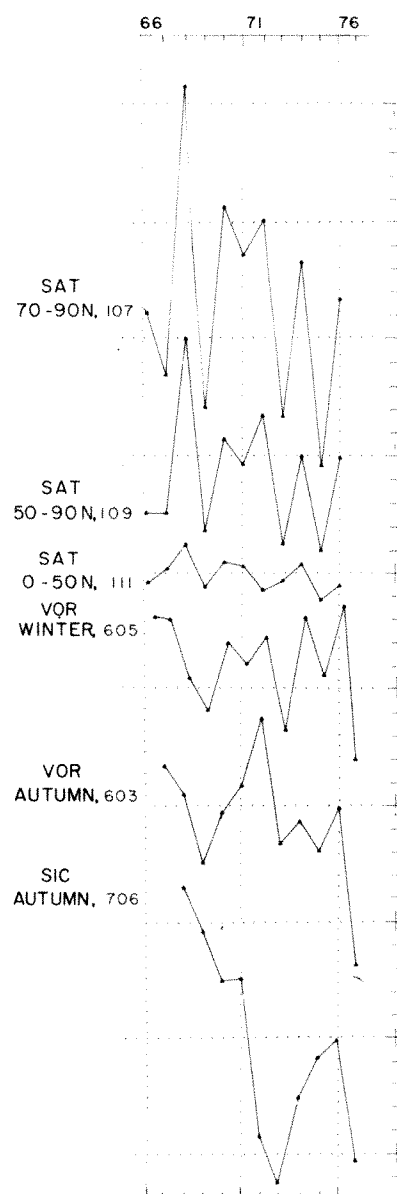
NASA, and DMSP polar orbiting satellites. Both curves show the total area of ice with a concentration greater than 1 octa.

Antarctic pack ice extent during selected seasons of the 1966–72 period was charted and studied by Streten<sup>36</sup>, Sissala *et al.*<sup>37</sup>, and Ackley and Keliher<sup>38</sup>. We reconstructed and measured additional charts using visible and infrared images from NOAA satellites. We also measured 1971–72 summer charts prepared by the US Navy. Monthly ice extents for the 1967–76 interval are greater than those reported by Treshnikov<sup>39</sup> for the late 1950s.

Comparison of curves 801 and 802 shows that the change of the mean extent of pack ice in November from 1972–76 roughly parallels the change in the average annual cover. Curve 802 for the end of November is, therefore, considered indicative of the average annual secular variations of pack ice cover between 1966 and 1972.

The November extent of pack ice increased between 1966 and 1972 but decreased from 1974 to 1976. Between 1967 and 1975 the Southern Hemisphere pack-ice variation (Fig. 3a, curves 801, 802) parallels the variation in Northern Hemisphere snow and ice cover (Fig. 3b, curve 704), as does the decrease in 1974 and 1975. The 1976–77 Antarctic ice season, however, continued to be light, while the snow coverage in the Northern Hemisphere was heavy. Thus, except for 1976–77, fluctuations in the surface extent of the

Fig. 5 Average climatic indices for autumn (September–November) and vortex area for winter (December–February). Symbols and scale as in Fig. 3.





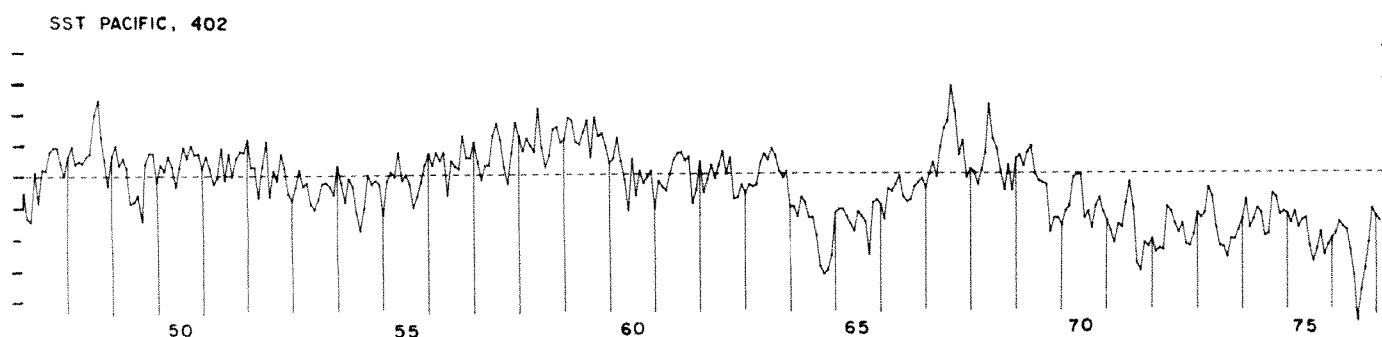


Fig. 6 Monthly departures of average sea surface temperatures in the north central Pacific from the 1947-66 mean. Vertical Scale in units of 0.2 °C.

cryosphere in the two hemispheres for the 1967-76 period, seem to be generally in phase.

## Discussion

The quality of individual records used in our study varies depending on the density of the station network, and frequency of observations. Also some of the data, for example, the upper atmosphere temperature and pressure readings, may have been influenced by gradual improvements of instrumentation. In general, observations made on the continents of both hemispheres are the most reliable. Temperature changes of the ocean surface, although of extreme importance in the climate system, are reliably known only over a fraction of the world's oceans, namely the north central Pacific, the central part of the North Atlantic and indirectly in the subpolar seas. Vast areas in the mid-latitudes of the Southern Hemisphere have remained virtually unobserved. Hence, conclusions on climate changes in the Southern Hemisphere and for the globe are much less reliable than those for the Northern Hemisphere. Also, our averaging techniques do not allow for descriptions of local trends in weather parameters which may be vastly different from mean zonal changes, and which are the most readily observable features by the population of affected regions<sup>51</sup>. Nevertheless, by comparing records of several independent climatic indices from different geographic areas and observing the fluctuations on a month to month basis, we were able to provide a cross check of most conclusions.

Summarising the data presented in Figs 3-5, the following observations can be made. (1) The year-to-year temperature fluctuations which compose most of the observed variance in our zonally average data, are in phase over most of the Northern

Hemisphere and also over parts of the Southern Hemisphere.

(2) The range of the year-to-year variability in most data sets is several times larger than the departure due to the long-term trends<sup>10,11,51</sup>. In this situation, reliable identification of trends or recognition of a trend reversal requires parallel observations made on different elements of the climate system over sufficiently long intervals<sup>52</sup>.

(3) The middle and low latitudes of the Northern Hemisphere show progressive oscillatory cooling throughout the recorded interval (Fig. 3a, Table 2). The high latitudes cooled until about 1965 and then show little change or warming.

(4) Pronounced cold episodes occurred globally in 1954-56; 1964-65, 1968, 1971-72, 1974 and 1976. The years 1958, 1959, 1970, 1973 and 1975 were comparatively warm. Several minima in curves 101 and 103 (Fig. 3) in the past 10 years reached the level of the single extreme recorded in the 1950s. Sea surface temperature minima were considerably lower in the 1970s than in the 1950s (Figs 3b, 6).

(5) From 1950 to 1975, the rate of cooling of most of the climatic indices in the Northern Hemisphere was between 0.1 and 0.2 °C per decade (Table 2). The North Atlantic sea surface and the free atmosphere of the northern middle and high latitudes show the largest drop in temperature. The drop in the surface air temperatures was relatively small and given the large magnitude of the fluctuations over this period, the trend may not even be statistically significant.

(6) The slope of the five-year running means of most indices from the middle and low latitudes of the Northern Hemisphere increased between 1971 and 1975 with respect to the 1966-70 pentad. During the same interval in the high latitudes the slope

Table 2 Average slope of overlapping pentadal means in the 1951-75 interval

Northern hemisphere			Average slope in °C per decade				
Curve	Parameter	Area	1955-60	1960-65	1965-70	1970-75	Average
301	ATM	0-90°N	NO	NO	-0.068	-0.324	-0.196
101	SAT	0-90°N	+0.088	-0.204	-0.068	-0.088	-0.068
202	ATM	65-90°N	+0.556	-0.944	-0.300	-0.208	-0.224
102	SAT	70-90°N	-0.760	-0.428	-0.184	+0.412	-0.240
201	ATM	35-90°N	+0.328	-0.316	-0.620	-0.160	-0.192
203	ATM	50-90°N	+0.476	-0.476	-0.584	-0.264	-0.212
105	SAT	50-90°N	-0.156	-0.072	-0.368	+0.324	-0.018
401	SST	N. Pac.	+0.304	-0.492	+0.188	+0.512	-0.128
501	SST	N. Atl.	-0.480	-0.200	-0.260	NO	-0.313
303	ATM	10-30°N	NO	NO	+0.144	-0.316	-0.086
103	SAT	0-50°N	+0.156	-0.244	+0.028	-0.220	-0.070
Southern hemisphere							
304	ATM	0-90°S	NO	NO	+0.116	+0.068	+0.092
307	ATM	60-90°S	NO	NO	+0.396	+0.468	+0.432
305	ATM	30-90°S	NO	NO	-0.016	+0.180	+0.082
306	ATM	10-30°S	NO	NO	+0.272	+0.104	+0.188
104	SAT	0-20°S	+0.168	-0.508	+0.072	-0.112	+0.095

NO, No data. Values are arithmetic averages of five departures between successive overlapping pairs of pentadal means. Observe that, for example, the value for 1955-60 was obtained from pentadal means for 1951-55, 1952-56, 1953-57, 1954-58, 1955-59 and 1956-60. Result was multiplied by 10 to show the decadal rates.

decreased or reversed (Table 2). In 1971–75 the sea surface in North Central Pacific and North Atlantic was significantly colder (Fig. 3a), snow cover and vortex area significantly larger (Fig. 3b), and average temperatures of the free atmosphere in the low and middle latitudes significantly lower than in the previous pentad (Fig. 3a and b). Record departures frequently occurred in 1976 and in early 1977. Our data do not show a reversal in the cooling of the Northern Hemisphere. The short-term recovery between 1973 and 1975 did not continue through 1976.

(7) The spring (March–May) surface-air temperatures during the last decade increased in the northern high latitudes but showed little change in the low and middle latitudes. At the same time, the spring vortex area and snow cover gradually expanded (Fig. 4). The autumn (September–November) surface-air temperatures in the last decade dropped significantly in the northern high latitudes, and, to a lesser degree in the low and middle latitudes. Autumn snow cover increased substantially while the autumn and winter vortex areas showed less change (Fig. 5).

(8) Reliable conclusions on temperature trends in the Southern Hemisphere are not yet possible since data are extremely sparse. Lack of information for the 30–60° latitude belt is a critical deficiency. In this belt, so poorly represented in our data set, satellite mapping has revealed extensive negative departure in the 1976 sea-surface temperatures with respect to the previous year<sup>42</sup>.

Our data from the Southern Hemisphere show cooling until the mid 1960s, then slight warming (Table 2). In high latitudes, south of 60°S, the upper air gradually warmed throughout the recorded interval. The pack-ice area expanded between 1967 and 1973, and decreased thereafter. According to Damon and Kunen<sup>40</sup>, the surface air in the Southern Hemisphere warmed between 1943 and 1974. Yamamoto *et al.*<sup>2</sup> found the 0–90°S surface air 0.03 °C cooler in the 1968–72 interval than in the pentad ending in 1962. Angell and Korshover<sup>17</sup> reported that the free atmosphere in the early 1970s was cooler than in the late 1950s. In all the mentioned studies, however, the reported temperature departures are very small, data variability very large and station density insufficient.

(9) There are parallels in the climate development of the two hemispheres. First, the atmosphere in the high latitudes warmed during the last decade; second, snow and pack ice expanded in 1972 and 1973 but decreased in 1974 and 1975; third, accelerated cooling took place in the early 1960s.

We have reported changes of selected climatic indices zonally averaged over large segments of the globe. Possible causes of the fluctuations have been discussed elsewhere<sup>1,16,43–45</sup>. Our zonally averaged indices have suppressed large spatial inhomogeneities which occur within any given latitude band. A coordinated investigation of these regional shifts of weather patterns, including the incidence of various weather extremes, will be our next objective.

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1. Yamamoto, R., Iwashima, T. & Hoshiai, M. *J. meteor. Soc., Japan* **53**, 482–486 (1975).
2. Yamamoto, R., Hoshiai, M. & Iwashima, T. *Arch. met. Geophys. Biokl. B* (in the press).
3. *World Weather Records* U.S. Dept. of Commerce, NOAA, Environmental Data Service.
4. *Monthly Climatic Data for the World* (U.S. Dept. of Commerce, NOAA, Environmental Data Service).
5. Cline, A. K. *Atmos. Tech.* **3**, 60–65 (1973).
6. Willett, H. C. *Proc. NOAA Climate Diagnostics Workshop*, 2201–2212, (U.S. Dept. of Commerce, NOAA, 1976).
7. Willett, H. C. *Continuity Proc. R. meteor. Soc.* 195–206 (1950).
8. Mitchell, J. M., Jr *Ann. N.Y. Acad. Sci.* **95**, 235–250 (1961).
9. Budyko, M. I. *Tellus* **21**, 611–619 (1969).
10. Reitan, C. H. *Quat. Res.* **4**, 25–38 (1974).
11. Brinkmann, W. A. R. *Quat. Res.* **6**, 355–358 (1976).
12. Dronia, H. *Met. Rdsch.* 166–174 (1974).
13. Dronia, H. *Beil. Berl. Wetterk.* 1–8 (1973).
14. Grosswetterlagen Europas, Deutscher Wetterdienst, Offenbach a.M.
15. Starr, V. P. & Oort, A. M. *Nature* **242**, 310–313 (1973).
16. Angell, J. K. & Korshover, J. *Mon. Weath. Rev.* **105**, 375–385 (1977).
17. Angell, J. K. & Korshover, J. *Proc. NOAA Climate Diagnostics Workshop*, 101–116, (U.S. Dept. of Commerce, NOAA, 1976).
18. Namias, J. *J. geophys. Res.* **75**, 565–582 (1970).
19. Namias, J. *Short Period Climate Variations, Collected Works of J. Namias 1934–1974*, 905 (University of California, San Diego, 1975).
20. McLain, D. R. *Proc. NOAA Climate Diagnostics Workshop*, 1201–1217 (U.S. Dept. of Commerce, NOAA, 1976).
21. Rodewald, M. *Beil. zur Berliner Wetterk.* 1 (1973).
22. Rodewald, M. *Beil. zur Berliner Wetterk.* 1–5 (1975).
23. Angell, J. K. & Korshover, J. *Mon. Weath. Rev.* **105**, 19–25 (1977).
24. Angell, J. K. & Korshover, J. *Mon. Weath. Rev.* (in the press).
25. Quiroz, R. S. *Proc. NOAA Climate Diagnostics Workshop*, 301–324 (U.S. Dept. of Commerce, NOAA, 1976).
26. Kukla, G. J. & Kukla, H. J. *Science* **183**, 709–714 (1974).
27. Kukla, G. J. *Proc. Symp. Meteorological Observations from Space: their Contribution to the First GARP Global Experiment*, 110–115 (NCAR, Boulder, Co., 1976).
28. Wiesnet, D. R. & Matson, M. *Mon. Weath. Rev.* **104**, 828–835 (1975).
29. Matson, M. & Wiesnet, D. R. *Proc. NOAA Climate Diagnostics Workshop*, 601–615 (U.S. Dept. of Commerce, NOAA, 1976).
30. Matson, M. *NOAA Tech. Mem. NWS* **84**, 24 (1977).
31. Sanderson, R. M. *Meteor. Mag.* **104**, 313–323 (1975).
32. *Monthly British Ice Charts* (Meteorological Office, Bracknell).
33. Haupt, I. & Kant, V. *Proc. Symp. Meteorological Observations from Space: their Contribution to the First GARP Global Experiment* 179–186 (NCAR, Boulder, Co., 1976).
34. Geb, M. *Ann. Met. N. F.* **12**, 80–83 (1977).
35. Gloersen, P., Wilheit, T. T., Chang, T. C., Nordberg, W. & Campbell, W. J. *Bull. Am. Met. Soc.* **55**, 1442–1448 (1974).
36. Streten, N. A. *Arch. Met. Geophys. Biokl. A22*, 119–134 (1973).
37. Sissala, J. F., Sabatini, R. R. & Ackerman, H. J. *Polar Res.* **16**, 367–373 (1972).
38. Ackley, S. F. & Kelher, T. E. *AIDJEX Bull.* **33**, 53–76 (1976).
39. Treshnikov, A. F. *Proc. Symp. Pacific-Antarctic Sciences*, 113–123 (1967).
40. Damon, P. E. & Kunen, S. M. *Science* **193**, 447–453 (1976).
41. Rogers, J. C. *Mon. Weath. Rev.* **104**, 985–993 (1976).
42. Strong, A. E. *Trans. Am. geophys. Union* **58**, 400 (1977).
43. Mitchell, J. M. *Quat. Res.* **6**, 481–493 (1976).
44. Broecker, W. S. *Science* **189**, 460–463 (1975).
45. Lamb, H. H. *Proc. R. Soc. Lond. A266*, 425 (1970).
46. Lamb, H. H. *Climat. Res. Unit Res. Publ.* 3 (1974).
47. Perry, A. H. *Weather* **29**, 451–455 (1974).
48. Painting, D. J. U.K. *Meteorological Office Scientific Paper* **35**, 22 (1977).
49. Wahl, E. W. & Bryson, R. A. *Nature* **254**, 45–46 (1975).
50. Flohn, H. *Climat. Change* **1**, 1–20 (1977).
51. van Loon, H. & Williams, J. *Mon. Weath. Rev.* **104**, 12, 365–380; 1003–1011; 1591–1596 (1976).
52. van Loon, H. & Williams, J. *Mon. Weath. Rev.* **105**, 5, 636–647 (1977).

# Conjugation proteins encoded by the F sex factor

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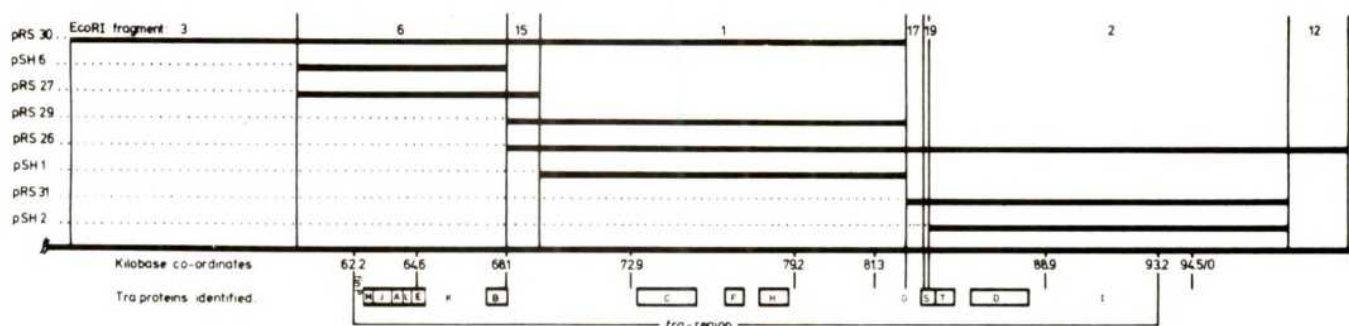
*Chimaeric plasmids carrying EcoRI fragments of the F sex factor have been used to identify proteins involved in conjugation and to assign them to tra cistrons. Most of these proteins are incorporated into the cell envelope and are individually regulated at the post-transcriptional level.*

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THE transfer of genetic material between bacteria by conjugation is an important evolutionary mechanism by which bacteria adapt to a changing environment, and also provides a model system for analysing cell-cell interactions. Conjugation is mediated by a variety of sex factors: we have chosen to work with the F sex factor whose genetics are the best known. The genes required for the synthesis of F pili, stabilisation of the mating aggregates, DNA transfer, and surface exclusion are contained in a single large transcriptional unit<sup>1</sup>. For simplicity this is called the 'tra operon'. In it some 13 cistrons have been genetically defined (see Fig. 1). Of these, nine (*traA*, *L*, *E*, *K*, *B*, *C*, *F*, *H* and part of *traG*) are



**Fig. 1** Map of the F transfer region. Horizontal bars represent the fragments of F cloned in the corresponding chimaeric plasmids. The origin of DNA transfer is to the right of the 62.2 kb coordinate<sup>14</sup>. Proteins which we have identified are boxed. The lengths of the boxes correspond to the lengths of DNA required to code for the proteins. Except for *traM* and *traS*, about which there remains some ambiguity, the order and location of the cistrons shown have been demonstrated<sup>4</sup>. The pRS chimaeric plasmids were cloned<sup>9</sup> using the plasmid vector pSC101 (ref. 23), and the pSH chimaeric plasmids were cloned using the plasmid vector ColE Amp (ref. 10a).

needed for the synthesis of F pili<sup>2-4</sup>. In addition, expression of the whole of *traG* is needed before F-carrying donor cells can form stable mating aggregates with F<sup>-</sup> recipients<sup>5</sup>. An additional three cistrons, *traM* (which is outside the *tra* operon), *traD* and *I*, are required for DNA transfer<sup>2-4</sup>. The *traS* and *T* cistrons convert F-carrying cells into poor recipients in conjugation with other F-carrying donors<sup>5,6</sup> (the surface exclusion phenomenon). It seems that transcription of the *tra* operon is promoted by the product of the positive control gene *traJ* (refs 7,8). *traJ* is itself regulated by a repressor complex, the products of the *finP* and *finO* genes<sup>8</sup>. The *finO* gene is missing in the F sex factor, and so F *tra* genes are normally fully expressed.

All these cistrons have been genetically mapped relative to the endonuclease *EcoRI* sites of F (ref. 4) and chimaeric plasmids expressing the *tra* cistrons have been cloned<sup>4,9</sup>. We have now used these chimaeric plasmids to make a comprehensive analysis of the protein products of the *tra* cistrons, as synthesised *in vitro* and in minicells. This identification of the conjugation proteins has also allowed us to examine their regulation and intracellular location.

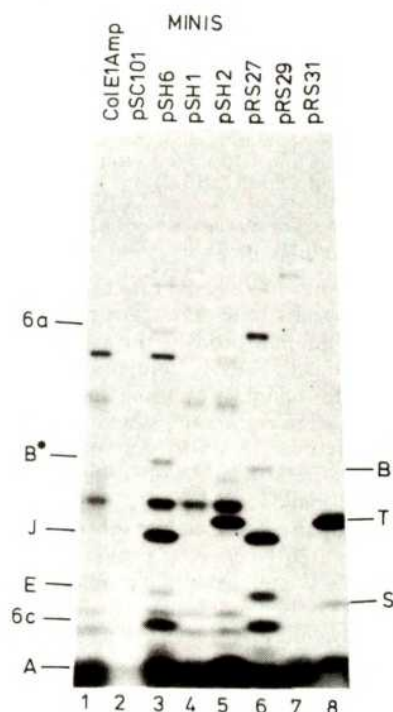
### Expression of *tra* cistrons by chimaeric plasmids

Two series of chimaeric plasmids have been constructed<sup>4,9</sup> from *EcoRI* fragments of F DNA. The pRS series was constructed using the plasmid vector pSC101 (ref. 10) and the pSH series using ColEIAmp (RSF2124)<sup>10a</sup>. Unlike F itself these plasmid DNAs segregate readily into minicells and can be purified in good yields. As shown in Fig. 1 the cloned DNAs of the pRS series each span one or more *EcoRI* sites in the *tra* region, whereas those of the pSH series do not. We were able to take advantage of this to detect possible hybrid peptides arising from read-through at the junctions between cloned and vector DNA. The chimaeric plasmids pRS27, pRS29 and pRS31 each carry approximately one third of the *tra* region, and each encodes relatively few *tra* proteins. These plasmids were used to identify the *tra* proteins as described below.

In our conditions, the only proteins synthesised in minicells were those directed by plasmid DNA which had segregated into the minicells. Minicells carrying chimaeric plasmids showed a linear increase in the incorporation of radioactively-labelled amino acids into proteins over the time period used. Analysis of these radioactive proteins by SDS polyacrylamide gel electrophoresis followed by autoradiography revealed the individual proteins encoded by the chimaeric plasmids, and the intensity of the bands corresponded to the net rates of synthesis.

Genetic complementation analyses<sup>4</sup> have demonstrated that the inserted DNA of all these chimaeric plasmids is expressed in the cell. Since only chimaeric plasmids carrying *EcoRI* fragment 6 (f6) possess the *tra* operon promoter, which is located between *traJ* and *traA* (ref. 1), the others must depend on transcription initiated from promoters on the plasmid vectors for expression of the inserted DNA. In minicells the chimaeric plasmids directed the synthesis of specific protein bands which were characteristic of

the cloned *EcoRI* fragment. Figure 2 shows that under our conditions, few proteins were encoded by the parent plasmids pSC101 and ColEIAmp and that additional proteins were synthesised when chimaeric plasmids were used as templates. With the



**Fig. 2** Proteins synthesised in minicells from plasmid templates and analysed by SDS polyacrylamide gel electrophoresis. The plasmids present in the minicells are indicated above individual tracks on the autoradiogram. The positions of protein species subsequently identified (see Fig. 3) are marked at the sides of the figure. Note that TraAp (tracks 3 and 6) is overshadowed by low molecular weight material on this autoradiogram and that pSC101-encoded proteins are only visible after longer exposures. No protein synthesis was detected in minicells not carrying any plasmid DNA. All bacterial strains were derivatives of the Su<sup>-</sup> minicell-producing strain DS410 (ref. 24). Minicells were purified by two successive sucrose gradient centrifugations<sup>25</sup> from 150 ml cultures grown to stationary phase in L broth<sup>26</sup>. The preparations contained less than one viable cell per 10<sup>4</sup> minicells. Minicell suspensions (6 × 10<sup>9</sup> minicells in 1.2 ml of 56/2 minimal medium<sup>26</sup> supplemented with glucose and thiamine) were incubated for 60 min at 37 °C followed by a further period of 60 min in the presence of 25 μCi of U-<sup>14</sup>C protein hydrolysate (Amersham, 55 mCi mM<sup>-1</sup>). The minicells were then pelleted by centrifugation, resuspended in 1 ml of L broth and incubated at 37 °C for a further 10 min. After washing with L broth, the minicells were resuspended in 100 μl of sample buffer<sup>27</sup>, heated at 90 °C for 2 min and centrifuged for 2 min in an Eppendorf 3200 table centrifuge; the supernatant was analysed by SDS gel electrophoresis using 11 to 20% acrylamide gradients followed by autoradiography (fluorography) as described<sup>6,27</sup>.



Table 1 The *tra* proteins

Cistron	No. of mutants affecting protein/No. of mutants tested*	Molecular weight of protein ( $\times 10^{-3}$ )†	Molar ratio‡		% bound to cell envelope¶
			<i>in vitro</i>	minicell	
<i>traM</i>	2/2	13	—	21	—
<i>traJ</i>	1/4	23.5	(100)	(100)	81
<i>traA</i>	3/3	13.7	141	26	96
<i>traL</i>	2/2	11	70	—	—
<i>traE</i>	2/2	19	22	17	88
<i>traK</i>	0/2	—	—	—	—
<i>traB</i>	2/2	29	7	12	67
<i>traC</i>	3/3	78	(20)	(20)	5–70
<i>traF</i>	3/3	25	49	17	75
<i>traH</i>	3/3	40	18	23	76
<i>traS</i>	3/3	18	7–170	22–58	76
<i>traT</i>	4/4	25	(112)	(112)	89
<i>traD</i>	2/2	77	2	3	5–70
<i>traI</i>	0/2	—	—	—	—

\*In most cases when any one mutant was tested, a single protein band disappeared from both the *in vitro* and the minicell products (representative results are given in Fig. 3). The primary exceptions were that TraMp was only detected in minicells, traLp was only detected *in vitro* and that protein 6c was synthesised at a lower rate *in vitro* (only) using pRS27 DNA carrying *traJ90*. Three further exceptions have also been included as mutants affecting a protein: TraMp and TraTp were synthesised at lower than normal rates by mutants carrying the mutations *traM226* (on pRS27) and *traT249* (on pRS31), respectively; TraBp disappeared and was replaced by two protein bands, one slightly smaller and the other slightly larger, when the mutation *traB269* on pRS27 was analysed.

†Molecular weights were determined relative to the following protein standards, whose molecular weights  $\times 10^{-3}$  are given in parentheses: RNA polymerase  $\beta'$  (165), RNA polymerase  $\beta$  (155), bovine serum albumin (68), catalase (60), aldolase (40), RNA polymerase  $\alpha$  (39), bovine chymotrypsinogen (25.7), lysozyme (14.3), myoglobin (17.2), cytochrome *c* (11.7) and F pilin (10.7). All proteins were from Boehringer (Mannheim) except F pilin. The molecular weights of the unidentified proteins shown in Fig. 3 were: 6a (tracks 1, 5–8, 9–11) 55,000; 6b (tracks 1, 5, 9–11) 24,000; 6c (tracks 1–11) 16,000; 2a (tracks 21, 23–25) 25,500. All except 2a were cell envelope associated.

‡The intensity of individual bands on autoradiograms was evaluated by densitometry. For each chimaera, these values were expressed relative to that determined for TraJp, TraCp or TraTp within the same track and after adjustment for the molecular weight. A total of one to four individual determinations were averaged. These determinations were highly reproducible for the major proteins. TraSp was usually synthesised *in vitro* at a rate 10 to 15-fold slower than TraTp but the results were occasionally so variable that we present only the range of values. To normalise between chimaeric plasmids, the relative value of TraCp to TraJp was determined with pRS30-carrying minicells, and this value used to correlate further to TraFp and TraHp. The relative incorporation into TraJp (pRS27) and TraTp (pRS31) was averaged from individual determinations and used for normalising the results with TraSp and TraDp.

¶Two autoradiograms, one of which is shown in Fig. 4, were evaluated by densitometry to estimate the percentage of each *tra* protein associated with the cell envelope fraction. The values for TraCp and TraDp varied in the two experiments and the range rather than the average is presented.

exception of polypeptides B and B\* (discussed below) the same additional protein species were detected regardless of the vector molecule. These additional proteins must therefore have been coded for by the cloned F DNA. Comparable results were also obtained with the other chimaeric plasmids shown in Fig. 1.

In an *in vitro* protein-synthesising system directed by purified plasmid DNA, only cistrons on f6 were expressed with equal efficiency from both pSC101 and ColE1Amp. The ColE1Amp-derived chimaeric plasmids pSH1 (*EcoRI* fragment f1) and pSH2 (f2) were poorer templates for protein synthesis *in vitro* than were the corresponding pSC101-derived plasmids pRS29 (f15, f1) and pRS31 (f17, f19, f2). We infer that the promoter adjacent to the *EcoRI* site in ColE1 is used more efficiently *in vivo* than in our *in vitro* system, and we interpret this as confirmation that the only strong promoter in the *tra* operon is on *EcoRI* fragment f6.

### Isolation of *tra* mutant chimaeras

To assign individual proteins to particular *tra* genes, we isolated a series of *tra* mutants derived from pRS27, pRS29 and pRS31, each mutant carrying a defined point mutation which drastically altered the structure of the corresponding protein. We used two methods to introduce mutations into the chimaeric plasmids. Defined amber and UGA *tra* mutations were moved by homozygosis from *Flac* to the chimaeric plasmids. Alternatively, the chimaeric DNAs were treated *in vitro* with hydroxylamine<sup>11</sup> and mutants were detected after transformation<sup>12</sup> into an appropriate *E. coli* strain. Only amber or non-leaky mutations were analysed further. Mutants derived by both homozygosis and mutagenesis were detected and characterised by genetic complementation tests<sup>4</sup>. We succeeded in isolating at least two independent non-leaky mutations in each of fourteen *tra* cistrons (see Table 1). Each mutation affected the expression of only one known cistron. The collection included amber mutations in the *tra* cistrons *J*, *E*, *B*, *C*, *F*, *H*, *T* and *D*. No attempt was made to isolate *traG* mutants, since the only available chimaeric plasmid carrying an intact *traG* cistron, pRS26, was too unstable for genetic manipulation. We were unsuccessful in attempts to isolate

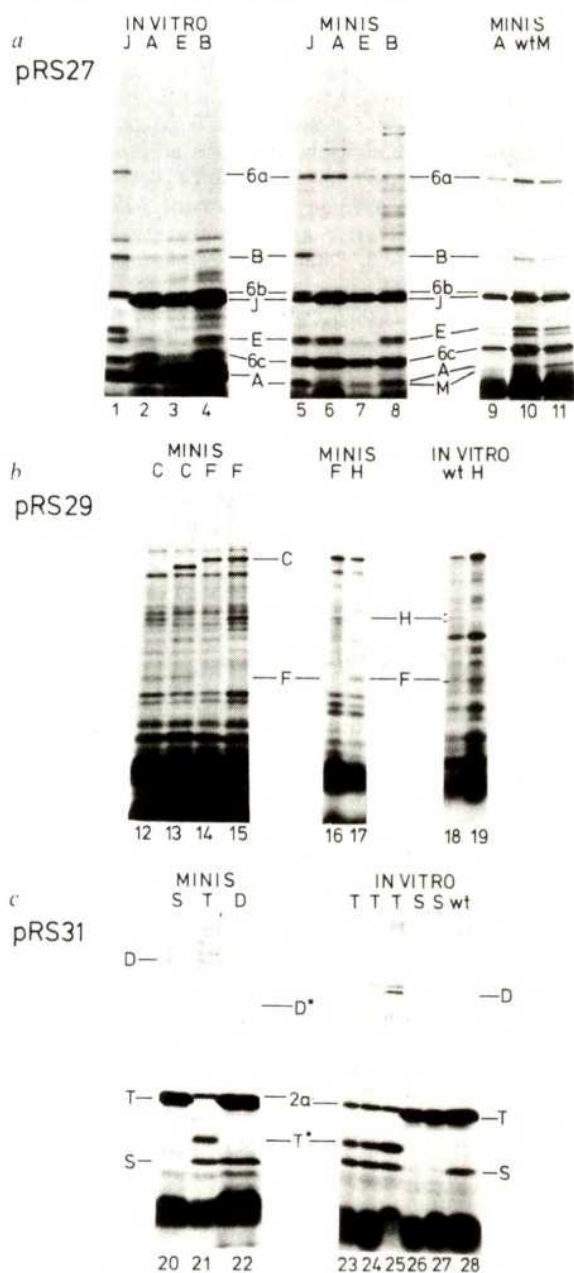
*finP* mutants of pRS27. All the mutant chimaeric plasmids were assigned numbers in the pBE series and some have already been described elsewhere<sup>4,6</sup>.

### Identification of conjugation proteins

Each assignment of a *tra* protein to a *tra* cistron reported here is based (with the exception of TraJp) on at least two independent mutations in that cistron resulting in the loss of, or change in a particular protein band. In essentially all cases, any one mutation had the same effect both *in vitro* and in minicells. Furthermore, there was a strict correlation between the cistron mutated and the protein affected, allowing us to identify proteins TraMp, TraJp, TraAp, TraLp, TraEp, TraBp, TraCp, TraFp, TraHp, TraSp, TraTp and TraDp. We now refer to these proteins as TraXp, rather than by our former nomenclature<sup>6</sup> (pTraX), since the latter may be confused with designations for plasmid chimaeras such as pRS27. Representative results are presented in Fig. 3. The molecular weights of the proteins thus identified, and the numbers of mutations affecting those proteins are summarised in Table 1. The molecular weights of the *tra* proteins have been correlated with physical mapping data (refs 4, 13 and Thompson, personal communication) to produce the map in Fig. 1. Thus our results both identify and map twelve conjugation (*tra*) proteins. Of the genetically defined cistrons on F which are involved in conjugation, only the products of *traK*, *G*, *I* and *finP* remain unidentified. We also detected other proteins encoded by the F factor for which no genes are yet known. The four major unassigned protein species (three encoded by *EcoRI* fragment 6 and labelled 6a, 6b and 6c and one encoded by fragment 2 and labelled 2a) are indicated in Fig. 3. Numerous other protein species encoded by *EcoRI* fragment 2 were also detected (see Fig. 3).

### Saturation of the map

In the following discussion we assume that *tra* cistrons do not overlap, that the origin of DNA transfer, *oriT*<sup>14</sup>, does not map



**Fig. 3** Identification of *tra* proteins. SDS polyacrylamide gel analysis of proteins synthesised from plasmids pRS27 (a), pRS29 (b) and pRS31 (c) carrying mutant cistrons as indicated above each gel track (wt, wild type). Minicell proteins (minis) were prepared as in Fig. 2. *In vitro* protein synthesis was as in ref. 18 but purified initiation factors were used. The *in vitro* system was prepared from the  $Su^+ F^-$  strain JC3272 (ref. 2) and the incubation was carried out in 50  $\mu$ l volumes containing 250  $\mu$ g washed ribosomes, 2  $\mu$ g IF1, 4  $\mu$ g IF2, 0.5  $\mu$ g IF3, 2.5  $\mu$ g chimaeric plasmid DNA, 10  $\mu$ l of S-150 fraction, and the other components described (in ref. 18). After 45 min incubation at 37 °C, the samples were treated with 50  $\mu$ g ml<sup>-1</sup> each of DNase I and RNase I at 37 °C for 1 min. 50  $\mu$ l of twice concentrated sample buffer<sup>24</sup> was added and the samples processed for SDS gel electrophoresis and fluorography as described<sup>6,27</sup>. Supercoiled plasmid DNA was purified from Triton X-100 cleared lysates<sup>28</sup> by CsCl-ethidium bromide centrifugation. After extracting the ethidium bromide into CsCl-saturated propan-2-ol, 30 to 70  $\mu$ g of DNA was concentrated into 100  $\mu$ l of buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) by ethanol precipitation. The figure shows a representative selection of mutations which led to the loss of the encoded gene product, as follows (\*Designates amber-suppressible mutations.): a, tracks 1,5, *traJ90\**; tracks 2,6,9, *traA1\**; tracks 3,7, *traE266\**; tracks 4,8, *traB269\**; track 11, *traM228\**; b, track 12, *traC271\**; track 13, *traC272\**; tracks 14,16, *traF13\**; track 15, *traF273\**; tracks 17,19, *traH88\**; c, tracks 20,26, *traS231\**; track 27, *traS232\**; track 23, *traT246\**; tracks 21,24, *traT247\**; track 25, *T248\**; track 22, *traD14\**. The following *tra* mutations also led to loss of the encoded protein: in pRS27, *A262*, *A278*, *E264\**, *E281\**, *B270\**, *L263* and *L311*; in pRS29, *C5\**, *F274\**, *H282\**, and *H283\**; in pRS31, *S233*, and *D285*. C\*, T\*, and D\*, amber peptides of TraCp, TraTp and TraDp.

within a cistron, and that the average molecular weight of an amino acid is 110. The *tra* cistrons to which we have assigned proteins account for a total of 10 kb of DNA. The corresponding cistrons all lie in the 31-kb region on F between the 62 and 93 kb coordinates<sup>4,13</sup>, and thus account for 33% of the coding capacity of this region. There is, however, a wide variation in the degree of saturation of the different *EcoRI* fragments. Thus the region on f6 to the right of *oriT* is almost completely saturated. In fact the space between *oriT* and a restriction endonuclease site in or near *traE* at 64.7 kb (R. Thompson, personal communication) is only sufficient to code for the defined proteins plus additional protein(s) of less than 20,000 daltons. This region includes the *finP* cistron<sup>15</sup>. Similarly, the available DNA between *traE* and *traB* requires that TraKp be smaller than 91,500 daltons. In contrast, the rest of the *tra* operon, on fragments 15, 1, 17, 19 and 2, includes large gaps with no known cistrons. Consequently the gene locations shown for this region in Fig. 1 are not nearly as well defined as those for f6.

### Most *tra* products are membrane proteins

We have analysed the intracellular location of the *tra* proteins synthesised in minicells. The cell envelope was separated from the cytoplasm and both fractions were analysed by SDS gel electrophoresis. Most of the *tra* proteins proved to be strongly associated with the cell envelope (Fig. 4 and Table 1). The exceptions were TraCp and TraDp for which the proportions associated with the cell envelope varied widely between experiments. Although the possibility exists that the minicell results are not directly applicable to whole cells, we infer that the following phenomena are cell membrane related: the regulation of the *tra* operon by TraJp, the synthesis of F pili involving TraAp, TraEp, TraBp, TraFp and TraHp, and the poor recipient ability mediated by TraSp and TraTp.

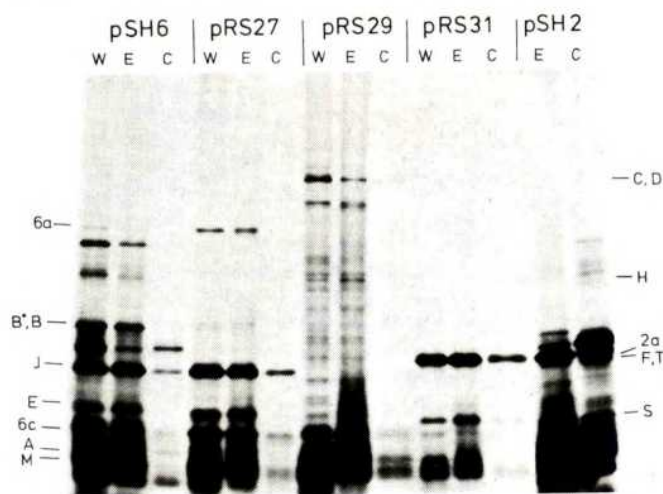
### Control by *traJ*

TraJp seems to be necessary for efficient transcription of the F factor *tra* operon in intact cells<sup>8</sup>. It is interesting that TraJp exhibited properties which are unusual for a control protein: it was associated with the cell envelope and it was synthesised at a very high rate. We anticipated that *traJ* mutants of pRS27 would exhibit pleiotropic reduction in the expression of the other *tra* operon proteins. This was not the case however. For four independent *traJ* mutants of pRS27 (one amber-suppressible (J90) (see Fig. 3, tracks 1,5) and three missense mutations), the rates of synthesis, *in vitro* and in minicells, of TraAp, TraEp and TraBp remained unchanged or even increased slightly. Thus whether TraJp is synthesised in a defective form, or is not synthesised at all, no regulatory effects on *tra* operon cistrons were detected. Since the *in vitro* system used may have been lacking in cell components involved in regulation, we attempted to reconstitute dependence on TraJp by adding cell-free extracts from F<sup>+</sup> cells to the *in vitro* system. These attempts were unsuccessful. Furthermore, no differences attributable to control by *traJ* were found when pRS27 or pRS27 *traJ* DNA templates were expressed in cell-free systems prepared from F<sup>-</sup>, F<sup>+</sup> *traJ*<sup>+</sup>, or F<sup>+</sup> *traJ*<sup>-</sup> cells. These negative results contrast with those obtained for several other genetic control proteins which do function *in vitro*<sup>16-18</sup>. Two possibilities could account for our results. If TraJp normally counteracts a mechanism which terminates transcription, the mechanism itself may be so labile that it is absent both *in vitro* and in minicells. Alternatively, TraJp might increase the binding specificity to the *tra* operon promoter of some cell component such as RNA polymerase. The absence of competing DNA (and promoters) *in vitro* and in minicells may then render this increased binding specificity superfluous.

### F pili

There is evidence that *traA* codes for the F pilus subunit protein F pilin<sup>19</sup>. We found that TraAp had the following properties. In minicells, it was synthesised at a much slower rate than *in vitro*, where it was the major *tra* product (Table 1 and Fig. 3a). It had an apparent molecular weight of 13,700 (Table 1 and Fig. 3, tracks





**Fig. 4** Intracellular location of *tra* proteins. Minicells containing radioactively labelled, plasmid-coded proteins, were fractionated into envelope and cytoplasmic components. The autoradiogram shows proteins from whole minicells (W), envelope (E) and cytoplasmic (C) fractions. The relevant plasmids are indicated above the tracks. The proteins encoded by pSH6 and pRS27 are indicated on the left of the figure whereas those encoded by pRS29 (C,H,F), pRS31 (D,T,S) and pSH2 (D,T) are indicated on the right. A minicell suspension (1.5 ml) prepared as in Fig. 2 was treated with  $50 \mu\text{g ml}^{-1}$  of lysozyme and 1 mM EDTA, pH 7.5 for 10 min at  $37^\circ\text{C}$ . The minicells were disrupted by sonication (six bursts of 30 s with cooling) and any unbroken minicells were removed by brief centrifugation. Minicell envelopes were pelleted at  $48,000 g$  for 60 min at  $2^\circ\text{C}$ . The cytoplasmic proteins in the supernatant were precipitated with trichloroacetic acid and resuspended in  $50 \mu\text{l}$  of sample buffer<sup>27</sup>. The membranes were resuspended in the same volume of sample buffer. Staining of the SDS gel before autoradiography revealed that distinctive cell envelope and cytoplasmic proteins were indeed enriched in the appropriate fractions.

9–11), and did not comigrate with F pilin obtained from purified F pili<sup>20</sup> (data not shown). F pilin has an apparent molecular weight of 10,700 (ref. 20). Thus if *traA* does code for F pilin, TraAp may be a precursor which is processed by a specific protease in the cell, losing a 3,000 dalton polypeptide to generate the F pilus subunit. However, this processing did not take place in minicells, where TraAp comigrated with the *in vitro traA* product and was associated with the cell envelope. We were unable to precipitate TraAp from *in vitro* reaction mixtures by purified F pilus antibodies (data not shown). It therefore remains possible that TraAp does not represent a precursor for F pilin, and that F pilin is coded for by a cistron other than *traA*.

### Map position of *traB*

Genetic complementation has shown that biologically active TraBp is synthesised both by pRS27 (carrying f6 and f15) and by pSH6 (carrying only f6)<sup>4</sup>. This leads to the conclusion that all of *traB* is located on f6. However, we found that no protein coded for by pSH6 comigrated with TraBp (Fig. 2). A new band, 1,000–2,000 daltons larger appeared instead (B\* in Fig. 2). We speculate that the last few carboxyterminal amino acids of TraBp are not essential for its biological function, and that the *traB* cistron possesses an *EcoRI* site very near its end. The additional 2,000–3,000 daltons in B\* (the TraBp analogue encoded by pSH6) would then be derived from a vector gene, probably that coding for colicin. The behaviour of one of the *traB* mutants supports this interpretation. We isolated an amber-suppressible mutant of pRS27 (carrying *traB270*). This coded for a TraBp amber peptide approximately five amino acids shorter than intact TraBp. This polypeptide retained 40% of its ability to complement *traB* mutants of the F sex factor, indicating that the carboxyterminal end of TraBp is not very important for its biological function. We have taken account of these considerations in locating *traB* as shown in Fig. 1.

### Surface exclusion

The poor recipient ability of F-carrying cells in conjugation with other F-carrying donor cells, is coded for by *traS* and *traT* (refs 5,6 and M. A., R. Thompson, S. Schwuchow, B. Kusecek and N. Willetts, manuscript in preparation). TraTp is an outer membrane protein present in up to 85,000 copies per cell<sup>6</sup>. This is of the same order of magnitude as that of the major *E. coli* outer membrane proteins I and II\*. It blocks the conversion of mating aggregates from an unstable to a stable form<sup>5,6</sup>. TraSp markedly reduces DNA transfer even within stable mating aggregates<sup>6</sup>.

The results presented here demonstrate that TraTp and TraSp are incorporated into the cell envelopes of minicells, that TraTp is one of the major products of the *tra* region and that TraSp is synthesised at widely varying rates relative to TraTp. These data also allow us to make a preliminary genetic mapping of *traS* and *traT*. The chimaeric plasmid pRS31 carries *EcoRI* fragments 17, 19 and 2 and directs the synthesis of both TraSp and TraTp (Fig. 2, track 8). The chimaeric plasmid pSH2 carries only *EcoRI* fragment 2 and codes for TraTp but not TraSp (Fig. 2, track 5). Thus while *traT* maps entirely or almost entirely on f2, *traS* probably maps at least partially on f17 or f19 as drawn in Fig. 1.

### Post-transcriptional regulation

There was great variation in the net rate of synthesis of individual *tra* operon proteins. Those with much the highest rate were TraAp, which was the main *in vitro* product, and TraTp. TraTp was synthesised at very high rates, both *in vitro* and in minicells, and it was the only *tra* operon product detectable on SDS gel electrophoresis of whole cell envelope proteins<sup>6</sup>. The cistrons coding for these two proteins are located at the beginning (*traA*) and towards the end (*traT*) of the *tra* operon (Fig. 1).

In contrast other proteins coded for by all three of the major *EcoRI* fragments of the *tra* region were synthesised at low rates. These include TraMp, TraEp, TraBp, TraCp, TraFp, TraHp, TraDp and the numerous unidentified proteins mainly coded for by fragment f1. All these proteins, except TraMp, are coded for by cistrons within the *tra* operon. They are always synthesised at low rates, irrespective of the vector or the size of the cloned DNA directing their synthesis. Comparable results for the same proteins were obtained whether transcription proceeded from the *tra* operon promoter (pRS27, pSH6 and pRS30) or from a promoter on the plasmid vector (pRS26, pSH1, pSH2 and pRS31). Thus cistrons transcribed after *traA* and before *traT* were expressed much less efficiently than *traA* and *traT* themselves (Table 1). Polar Mu-1 insertions in the *tra* operon have demonstrated that it contains no strong secondary promoters<sup>1</sup>. Thus the increased rate of synthesis of TraTp relative to the gene products of promoter proximal cistrons must result from regulation occurring at the post-transcriptional level. Since this control was not dependent on any particular promoter, we infer that the rate of translation is determined by the nucleotide sequence of the primary transcript. This conclusion adds another case to the list (see refs 21–23 for example) of recently reported examples of post-transcriptional rate determination in bacteria.

### Conclusions

Previous genetic analyses of *tra* cistrons on the F sex factor led to the present, relatively advanced, understanding of their regulation and biological function<sup>5</sup>, but yielded no information on the proteins involved. The genetic results are now complemented by the identification presented here of 12 of the *tra* cistron gene products. These results have facilitated the construction of a physical map showing the size and location of known *tra* cistrons, they have indicated the existence of numerous unidentified *tra* cistrons and proteins, and they have enabled us to begin an investigation at the biochemical level into the roles of these proteins in bacterial conjugation.

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1. Helmuth, R. & Achtman, M. *Nature* **257**, 652-656 (1975).
2. Achtman, M., Willetts, N. & Clark, A. J. *J. Bact.* **106**, 529-538 (1971).
3. Willetts, N. *Genet. Res., Camb.* **21**, 205-213 (1973).
4. Achtman, M. *et al.* *J. Bact.* (in the press).
5. Achtman, M. & Skurray, R. A. in *Microbial Interactions: Receptors and Recognition, Series B* (ed. Reissig, J. L.) **3**, 234-279 (Chapman & Hall, London, 1977).
6. Achtman, M., Kennedy, N. & Skurray, R. A. *Proc. natn. Acad. Sci. U.S.A.* **74**, (in the press).
7. Finnegan, D. & Willetts, N. *Molec. gen. Genet.* **127**, 307-316 (1973).
8. Willetts, N. *J. molec. Biol.* **112**, 141-148 (1977).
9. Skurray, R. A., Nagaishi, H. & Clark, A. J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 64-68 (1976).
10. Cohen, S. N., Chang, A. C. Y., Boyer, H. W. & Helling, R. B. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3240-3244 (1973).
- 10a. So, M., Gill, R. & Falkow, S. *Molec. gen. Genet.* **142**, 239-249 (1975).

11. Humphreys, G. O., Willshaw, G. A., Smith, R. & Anderson, E. S. *Molec. gen. Genet.* **145**, 101-108 (1976).
12. Cohen, S. N., Chang, A. C. Y. & Hsu, L. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2110-2114 (1972).
13. Davidson, N., Deonier, R. C., Hu, S. & Ohtsubo, E. *Microbiology-1974*, 56-65 (American Society for Microbiology, Washington, DC, 1975).
14. Guyer, M. S., Davidson, N. & Clark, A. J. *J. Bact.* **131**, 970-980 (1977).
15. Willetts, N., Maule, J. & McIntire, S. *Genet. Res., Camb.* **26**, 255-263 (1976).
16. Ponta, H., Rahmsdorf, H. J., Pai, S. H., Herrlich, P. & Schweiger, M. *Molec. gen. Genet.* **134**, 29-38 (1974).
17. Zubay, G., Chambers, D. A. & Cheong, L. C. in *The Lactose Operon* (ed. Beckwith, J. R. & Zipser, D.) 375-391 (Cold Spring Harbor Laboratory, 1970).
18. Herrlich, P., Rahmsdorf, H. J., Pai, S. H. & Schweiger, M. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1088-1092 (1974).
19. Minkley, E. G., Jr., Polen, S., Brinton, C. C., Jr. & Ippen thier, K. *J. molec. Biol.* **108**, 111-121 (1976).
20. Helmuth, R., thesis, Freien Universitaet Berlin (1977).
21. Herrlich, P. & Schweiger, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3386-3390 (1976).
22. Stettin, J. A., Waldba, A. J., Laughrea, M. & Moore, P. B. *Nucleic Acids Res.* **4**, 1-15 (1977).
23. Swebelius-Singer, B. & Gould, L. M. *J. molec. Biol.* **103**, 627-646 (1976).
24. Dougan, G. & Sherratt, D. *Molec. gen. Genet.* **151**, 151-160 (1977).
25. Roozen, K. J., Fenwick, R. G., Jr. & Curtiss, R., Jr. *J. Bact.* **107**, 21-33 (1971).
26. Achtman, M. *J. Bact.* **123**, 505-515 (1975).
27. Lugtenberg, B., Meijers, J., Peters, R., van der Hoek, P. and von Alphen, L. *FEBS Lett.* **58**, 254-258 (1975).
28. Clewell, D. B. & Helinski, D. R. *Proc. natn. Acad. Sci. U.S.A.* **62**, 1159-1166 (1969).

# letters to nature

## Superfluid $^3\text{He A}$ is a liquid ferromagnet

THE superfluid A phase of liquid  $^3\text{He}$  (see for example refs 1, 2) is often said to display orbital ferromagnetism<sup>3</sup>. This term generally seems to be meant metaphorically and to imply that this phase possesses a finite total orbital angular momentum in equilibrium<sup>3</sup>. I point out here that superfluid  $^3\text{He A}$  is in fact quite literally ferromagnetic, with a spontaneous magnetic moment which, although extremely small on the scale of ordinary ferromagnets ( $\sim 10^{-11}$  Bohr magnetons per atom) is probably measurable and may indeed have some interesting experimental implications.

The basic mechanism is that the Cooper pairs in  $^3\text{He A}$  (which for present purposes may be thought of as giant diatomic molecules) possess a relative orbital angular momentum<sup>3</sup> of  $\hbar$  directed along the so-called  $\mathbf{l}$  vector<sup>2</sup>; in a way familiar in the theory of diatomic molecules<sup>4</sup>, a small part of this is transferred, via the electronic-rotational coupling, into motion of the electrons around the nuclei, thereby producing a finite magnetic moment for each pair. In contrast to the thermally disoriented diatomic molecules in an ordinary gas, however, all the Cooper pairs in  $^3\text{He A}$  have the same direction of orbital angular momentum, so the system behaves as a ferromagnet.

I have attempted to calculate the order of magnitude of the spontaneous magnetic moment so produced by a technique closely analogous to that used in ref. 5 to estimate the parity-violating electric dipole moment of  $^3\text{He B}$ ; that is, I first calculate the magnitude  $\mu(R)$  of the orbital magnetic moment of a single  $^3\text{He}$  dimer with nuclear separation  $R$  and relative orbital angular momentum  $\hbar$ , then multiply by the square of the radial part of the Cooper-pair wave function,  $|F(R)|^2$ , and integrate over all  $R$ . (The average over the angular wave function gives unity.) For  $F(R)$  I make the same ansatz as in ref. 5, namely  $F(R) = AR^{-1} \sin k(R-R_0)$ , where  $R_0$  is the 'hard-core radius' ( $\sim 2.5 \text{ \AA}$ ).  $A$  is fitted from the experimental dipole energy<sup>2</sup>  $g_D(T)$  and I assume  $k \gtrsim k_F$  ( $k_F$  = Fermi wavevector). This gives for the spontaneous magnetic moment per unit volume along  $\mathbf{l}$  in a uniform sample

$$M(T) \cong \frac{g_D(T)R_0^2}{\gamma^2 \hbar^2} \int_{R_0}^{\infty} \mu(R) \cdot 2 \sin^2 k(R-R_0) dR \quad (1)$$

To estimate the quantity  $\mu(R)$  I first note that if a single neutral atom of mass  $M$ , in a slightly deformed electronic S-state, is constrained to rotate about the origin with its nucleus at a distance  $R/2$  and with angular momentum  $\hbar/2$ , then the orbital magnetic moment is given, to lowest order in the deformation, by the expression  $-2R^{-1}P(R)(m/M)|\mu_B|$ , where  $m$  is the electron mass,  $\mu_B$  the Bohr magneton and  $P(R)$  the polarisation of the atom in the outward direction in units of  $e(\equiv -|e|)$ . I shall now assume that for a  $^3\text{He}$  dimer with nuclear separation  $R$  and relative angular momentum  $\hbar$  the correct order of magnitude, at least, of  $\mu(R)$  may be obtained by approximating the electronic wave function by an antisymmetrised product of  $1\sigma_g$  and  $1\sigma_u$  SCF-MO single-particle functions as in for example ref. 6, calculating  $P(R)$  for each atom separately and summing over the two atoms. For the SCF-MO functions I use the forms computed numerically in ref. 6. Since there are only four relevant data points and the corresponding values of  $\mu$  by no means fall on a smooth curve, any attempt to determine the general form of  $\mu(R)$  in this way is somewhat hazardous, but if we arbitrarily try to fit it to the intuitively plausible formula ( $a_0$  = Bohr radius)

$$\mu(R) = |\mu_B| \left( \frac{m}{M} \right) \left( \frac{a_0}{R} \right) C_0 \exp\{-\lambda(R-R_0)/a_0\} \quad (2)$$

then the 'best' values are probably  $C_0 \cong -1.1 \times 10^{-3}$ ,  $\lambda \cong 1.4$ .

It is convenient to express the final result for the spontaneous moment  $\mathbf{M}(T)$  in terms of an equivalent field  $\mathbf{H}_{eq}(T) \equiv \mathbf{M}(T)/\chi_p$ , where  $\chi_p$  is the normal-state paramagnetic susceptibility (not the total susceptibility). Note that 1 G is equivalent to about  $5 \times 10^{-10} \mu_B$  per atom. Combining equations (1) and (2) and substituting the experimental value<sup>2</sup> of  $g_D(T)$  for pressures near the melting curve and  $T/T_c \gtrsim 0.7$  ( $T_c$  = critical temperature of  $^3\text{He A}$ ) we find in this region

$$\mathbf{H}_{eq}(T) = H_l(1-T/T_c)\mathbf{l}, \quad H_l \cong -0.02 \dot{F}(k) \text{ G} \quad (3)$$

where  $\dot{F}(k) \cong (1+2k_F^2/k^2)^{-1}$ . Thus I tentatively estimate  $|H_l| \sim 10-20 \text{ mG}$ ; however, in view of the uncertainties in both the chemical and the many-body parts of the calculation this should be regarded as at best a crude order of magnitude.

A spontaneous magnetisation of this order should be detectable, probably most easily by observing its effect on the orientation of the  $\mathbf{l}$ -vector (as measured, for example, by ultrasonic

attenuation) at either low or ultra-high external fields (J. C. Wheatley, D. M. Lee, personal communications). (Note that unlike previously known orientation effects, this one is sensitive to the polarity of the field.) Its observation would have a number of interesting implications. (1) It would provide the first direct evidence that invariance under both space inversion and time reversal is spontaneously broken in  $^3\text{He A}$ . (2) The observed order of magnitude would provide some measure of the credibility of the above method of calculating 'chemical' effects in superfluid  $^3\text{He}$ , and hence for example of the theoretical predictions of ref. 5. (3) There should be related effects in  $^3\text{He B}$ , for example, an interaction of the nuclear spins with the electronic orbital moments<sup>7</sup> which will shift the spin-orbit rotation angle<sup>2</sup> slightly away from the 'magic angle'  $\cos^{-1}(-1/4)$ . (4)  $^3\text{He A}$  would presumably constitute the first ferromagnetic liquid known in nature.

I thank Bill Truscott (who first suggested to me the possibility of orbital magnetic effects in superfluid  $^3\text{He}$ ), John Wheatley, Richard Webb and Dave Lee for helpful discussions, and Kathy Levin and her colleagues in the Solid State Theory group at the James Franck Institute for their hospitality.

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1. Wheatley, J. C. *Rev. mod. Phys.* **47**, 415-470 (1975).
2. Leggett, A. J. *Rev. mod. Phys.* **47**, 331-414 (1975).
3. Anderson, P. W. & Morel, P. *Phys. Rev.* **123**, 1911-1928 (1961).
4. Wick, G. C. *Phys. Rev.* **73**, 51-57 (1948).
5. Leggett, A. J. *Phys. Rev. Lett.* **39**, 587-590 (1977).
6. Kestner, N. R. *J. Chem. Phys.* **48**, 252-257 (1968).
7. Anderson, P. W. & Varma, C. M. *Nature* **241**, 187-189 (1973).

## Positions of galactic X-ray sources: $0^\circ < l'' < 20^\circ$

PRECISE (20-25'') positions of six X-ray sources located in the galactic bulge, GX1+4, GX9+9, GX3+1, GX13+1, G%13+1 and GX17+2 are reported here. The data were taken as part of the survey of the galactic plane performed with the SAS-3 rotating modulation collimators<sup>1-4</sup>. Previously proposed optical counterparts for three of these sources (GX1+4, GX9+9, and GX17+2) lie within our error circles. The positions, error radii, and

intensities (2-11 keV) determined for the sources are given in Table 1. We compare our results with those determined with previous sounding rocket and satellite experiments in Fig. 1. Proposed optical and radio candidates are also included. Finding charts for the six sources are given in Fig. 2.

The status of each source with regards to optical or radio identifications is as follows:

**GX1+4 (2S1728-247):** A candidate for this source was suggested by Glass and Feast<sup>5</sup>, who detected it first in the infrared (1.25-2.2  $\mu\text{m}$ ) and then optically, where nearly all the emission is in lines, primarily H $\alpha$ . The unusual nature of this object has been verified in subsequent work<sup>6,7</sup>, and its existence in the small Copernicus (K. Mason, personal communication in ref. 6) and SAS-3 X-ray error boxes leaves little doubt about the identification.

**GX9+9 (2S1728-169):** An optical candidate for this source has been suggested by Davidsen, Malina, and Bowyer<sup>8</sup> on the basis of the Copernicus<sup>9</sup> and Uhuru<sup>9</sup> positions (see Fig. 1). It has a  $B$  magnitude of 16.4 with an ultraviolet excess ( $U-B = -0.5$ ). The object lies 10'' from the position determined with the SAS-3 RMC, well within the error circle. Recently, emission lines of He II  $\lambda 4686$  and the C III, N III blend at 4640-4650 have been observed<sup>10</sup> in this candidate. The substantial reduction in X-ray error box area and the detection of emission lines common in optical counterparts of X-ray sources make the identification certain.

**GX3+1 (2S1744-265):** A very precise position for this source has been determined with a pair of lunar occultations<sup>11</sup>. The centre of our error circle is within 3'' of the centroid of the occultation determination. A weak radio source (G. K. Miley, personal communication in ref. 11) lies near the occultation position and is consistent with the SAS-3 position. There are no optical candidates for the source<sup>11,12</sup>.

**GX9+1 (2S1758-205):** There are numerous position determinations for this source<sup>8,9,13-15</sup> (see Fig. 1) but no optical candidates<sup>12,13,16</sup>. A weak radio source lies nearby<sup>17</sup>, but is excluded as a candidate by the X-ray positions.

**GX13+1 (2S1811-171):** There are currently no optical or radio candidates for this source, although searches have been made in both wavelength bands<sup>6,18</sup>.

**GX17+2 (2S1813-140):** A variable radio source was found by Hjellming and Wade<sup>19</sup> in the early X-ray error

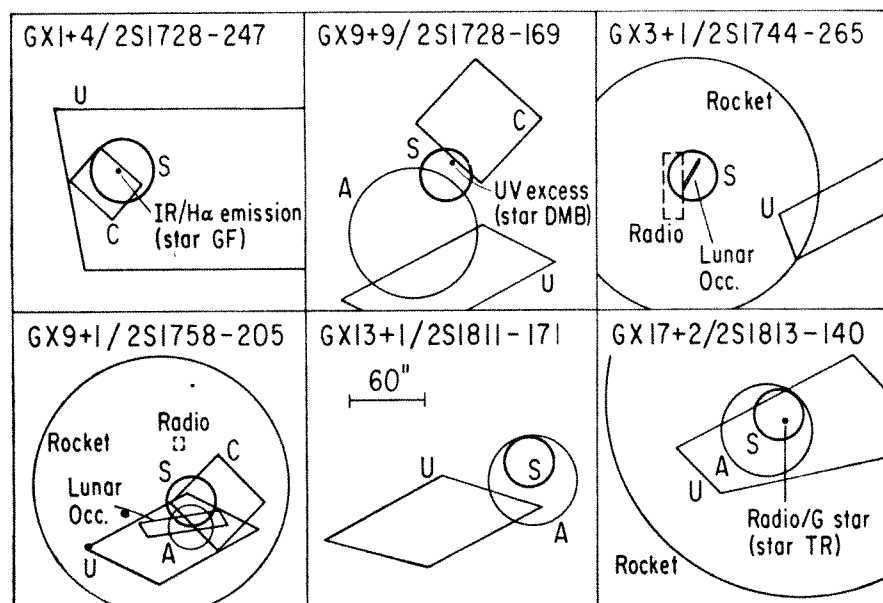


Fig. 1 The regions containing X-ray sources reported in this letter. The indicated scale factor applies to all regions. Optical and radio candidates are discussed in the text. X-ray error boxes are from the SAS-3 (S, this work); Uhuru (U, ref. 27); Ariel V (A, ref. 14), and Copernicus (C, ref. 8, and K. Mason, personal communication in ref. 6) satellites and from lunar occultations<sup>11,13</sup> and rocket<sup>15</sup> experiments.

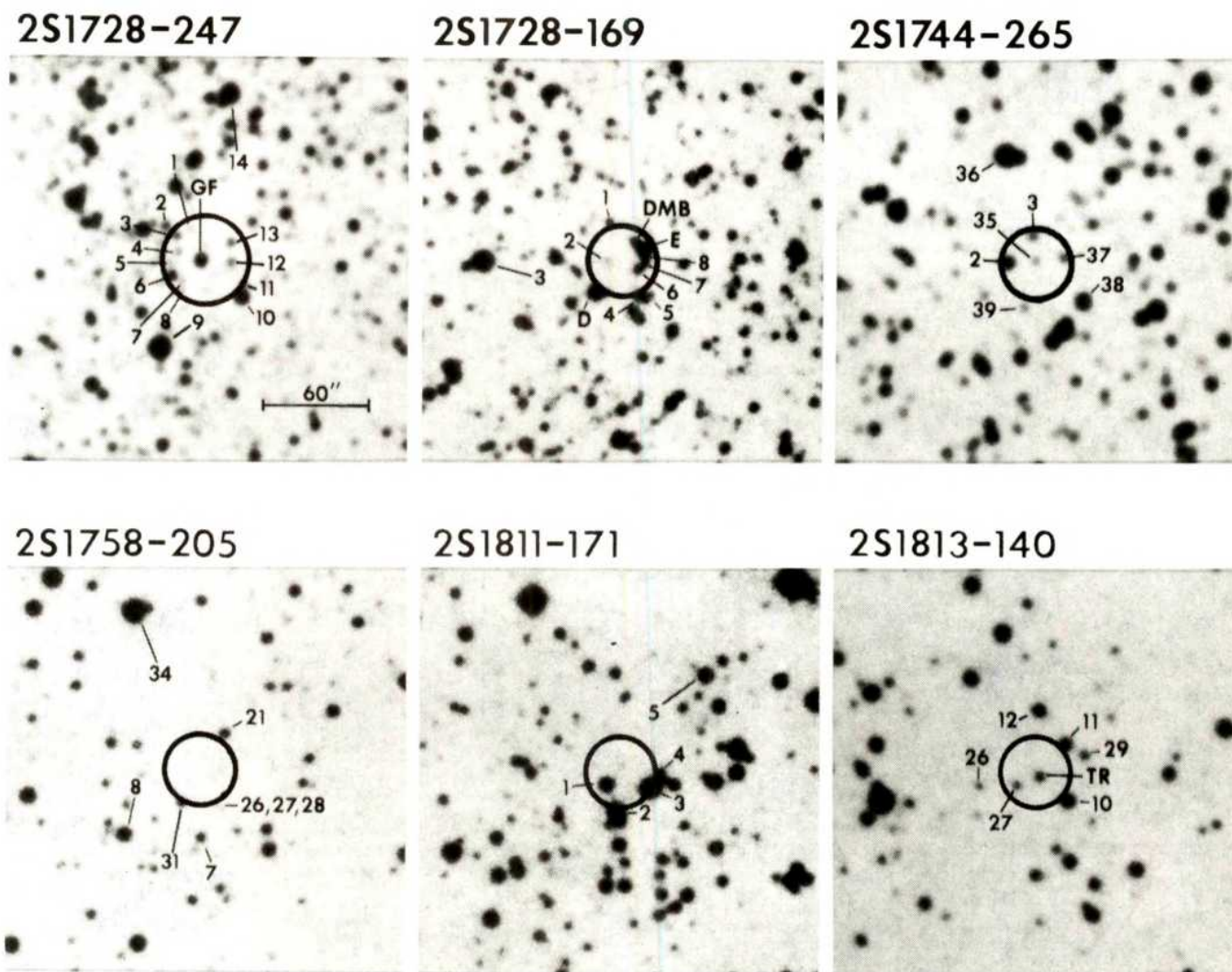


Fig. 2 Finding charts for the SAS-3 positions. The charts are taken from the Palomar Sky Survey red plates (National Geographic Society). North is up and east is to the left. The indicated scale factor applies to all charts. Stars are numbered to ease communication between observers; numbering and lettering schemes of previous observers have been adopted. The proposed identifications of Glass and Feast (GF, ref. 5), Davidsen, Malina, and Bowyer (DMB, ref. 6) and Tarengi and Reina (TR, ref. 21) are indicated.

Table 1 Celestial positions

SAS-3 designation	Other designations*	Position (1950)		$\mu^{\text{II}}$ $b^{\text{II}}$	Error radius (90%)	Flux density† (2–11 keV)	Comments‡
		$\alpha$	$\delta$				
2S1728–247	GX1+4 GX2+5 4U1728–24	17 h 28 min 57.4 s 262.2392	–24° 42' 42'' –24.7117	1.9 4.8	25''	55 $\mu\text{Jy}$	Optical counterpart
2S1728–169	GX9+9 4U1728–16	17 28 50.4 262.2100	–16 55 30 –16.9250	8.5 9.0	20	230	Optical counterpart
2S1744–265	GX3+1 4U1744–26	17 44 49.0 266.2042	–26 32 51 –26.5475	2.3 0.8	20	400	Possible radio candidate
2S1758–205	GX9+1 4U1758–20	17 58 33.3 269.6388	–20 31 44 –20.5289	9.1 1.2	20	480	
2S1811–171	GX13+1 4U1811–17	18 11 37.2 272.9050	–17 10 16 –17.1711	13.5 0.1	20	325	
2S1813–140	GX17+2 4U1813–14	18 13 11.2 273.2967	–14 03 10 –14.0528	16.4 1.3	20	490	Radio/optical candidate

\*Refs 25–28.

†Averaged over the observation and accurate to  $\sim 10\%$ . 1.0  $\mu\text{Jy}$  corresponds to  $2.2 \times 10^{-11} \text{ erg s}^{-1} \text{ cm}^{-2}$  (2–11 keV).  $I_{\text{crab}} = 1,060 \mu\text{Jy}$  (see refs 1, 2).

‡See text for discussion and references.



boxes<sup>15,20</sup>. Tarengi and Reina<sup>21</sup> pointed out that there is only one star visible on the Palomar Sky Survey within the radio error box. Hoag and Weisberg<sup>22</sup> made a deep red survey of the region and found one additional faint M star which lies in the SAS-3 error circle, but none in the radio error box to a limit of  $R \sim 20$  mag. The identification with the Tarengi and Reina candidate depends on the assumption that the radio and X-ray sources are coincident. The optical object has a normal G type spectrum<sup>6</sup> and shows no evidence<sup>23</sup> of the reported 30 min X-ray period<sup>24</sup>.

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1. Bradt, H. V. *et al.* *Nature* **269**, 21–25 (1977).
2. Doxsey, R. E., Apparao, K. M. V., Bradt, H. V., Dower, R. G. & Jernigan, J. G. *Nature* **269**, 112–116 (1977).
3. Bradt, H. V., Apparao, K. M. V., Dower, R. G., Doxsey, R. E., Jernigan, J. G. & Markert, T. H. *Nature* **269**, 496–497 (1977).
4. Jernigan, J. G., Apparao, K. M. V., Bradt, H. V., Doxsey, R. E. & McClintock, J. E. *Nature* **270**, 321–323 (1977).
5. Glass, I. S. & Feast, M. W. *Nature phys. Sci.* **245**, 39–40 (1973).
6. Davidsen, A., Malina, R. & Bowyer, S. *Astrophys. J.* **203**, 448–454 (1976).
7. Davidsen, A., Malina, R. & Bowyer, S. *Astrophys. J.* **211**, 866–871 (1977).
8. Willmore, A. P. *et al.* *Mon. Not. R. astr. Soc.* **169**, 7–23 (1974).
9. Giacconi, R., *et al.* *Astrophys. J. Suppl.* **27**, 37–64 (1974).
10. Charles, P. A., Thorstensen, J. R. & Bowyer, S. *IAU Circ. No.* 3096 (1977).
11. Janes, A. F., Pounds, K. A., Ricketts, M. J., Willmore, A. P. & Morrison, L. V. *Nature* **244**, 349 (1973).
12. Kunkel, W. *et al.* *Astrophys. J. Lett.* **161**, L169–L172 (1970).
13. Davison, P. J. N. & Morrison, L. V. *Mon. Not. R. astr. Soc.* **178**, 53p–56p (1977).
14. Wilson, A. M., Carpenter, G. F., Eyles, C. J., Skinner, G. K. & Willmore, A. P. *Astrophys. J. Lett.* **215**, L111–L115 (1977).
15. Schnopper, H. W. *et al.* *Astrophys. J. Lett.* **161**, L161–L167 (1970).
16. Murrin, P. *et al.* *Mon. Not. R. astr. Soc.* **169**, 25–34 (1974).
17. Zaumen, W., Murthy, G. T., Rappaport, S., Hjellming, R. M. & Wade, C. M. *Nature* **235**, 378–379 (1972).
18. White, N. E., Mason, K. O., Sanford, P. W., Johnson, H. M. & Catura, R. C. *Astrophys. J.* (submitted).
19. Hjellming, R. M. & Wade, C. M. *Astrophys. J. Lett.* **168**, L21–L24 (1971).
20. Tananbaum, H., Gursky, H., Kellogg, E. & Giacconi, R. *Astrophys. J. Lett.* **168**, L25–L28 (1971).
21. Tarengi, M. & Reina, C. *Nature phys. Sci.* **240**, 53–54 (1972).
22. Hoag, A. & Weisberg, J. M. *Astrophys. J.* **209**, 908–911 (1976).
23. Margon, B. *Astrophys. J.* (in the press).
24. White, N. E., Mason, K. O., Huckle, H. E., Charles, P. A. & Sanford, P. W. *Astrophys. J. Lett.* **209**, L119–L124 (1976).
25. Lewin, W. H. G., Ricker, G. R. & McClintock, J. E. *Astrophys. J. Lett.* **169**, L17–L21 (1971).
26. Hawkins, F. J., Mason, K. O. & Sanford, P. W. *Nature phys. Sci.* **241**, 109–111 (1973).
27. Forman, W. *et al.* *Astrophys. J. Suppl.* (in the press).
28. Bradt, H., Naranan, S., Rappaport, S. & Spada, G. *Astrophys. J.* **152**, 1005–1013 (1968).

## Hollow meteor trains

ENDURING meteor trains that appear at a height of about 85 km as double lines of light have been reported for more than a century, the first record being Newton's<sup>1</sup> in 1869. Although the dual appearance, which has been seen in the telescope within a few seconds of train formation and by the naked eye after about 1 min, is not due to the occurrence of separate trains<sup>2</sup>, no explanation has yet been offered for the phenomenon. The peculiar formation almost certainly results from the expanding meteor train assuming the shape of a hollow cylinder<sup>3</sup> and it is shown here that this behaviour is to be expected on the basis of recent<sup>4,5</sup>

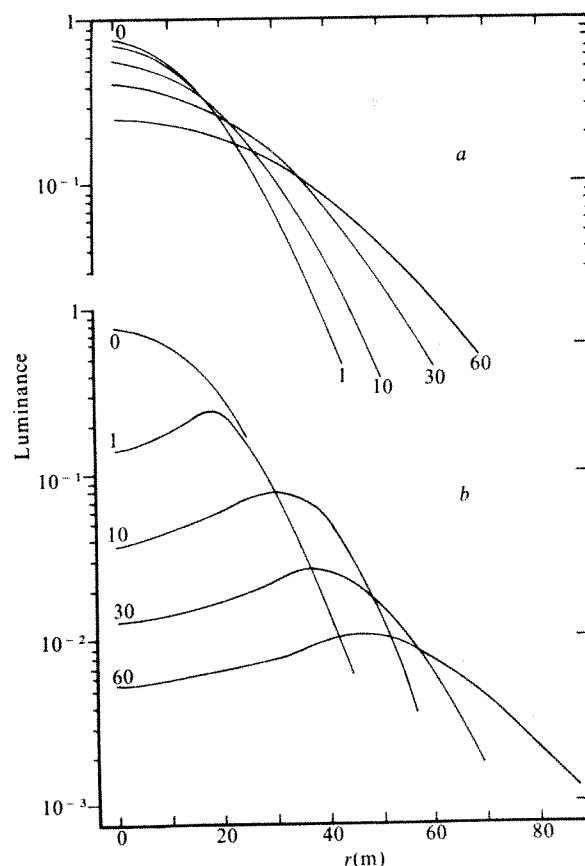


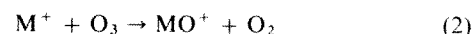
Fig. 1 Train surface brightness profiles for meteors of 30 and 60 km s<sup>-1</sup> at various times (s) after train formation;  $r$  is distance from train axis. Ordinate is normalised luminance. Solutions refer to meteoroid mass of 30 g zenith distance 30° corresponding to meteors of visual magnitudes approximately -3 and -6 at (a) 30 and (b) 60 km s<sup>-1</sup> respectively.

models of the mechanism of long enduring train luminosity.

An ablating meteoroid deposits Na atoms (meteoric Na abundance  $\sim 0.7\%$  see ref. 6) in a column some tens of kilometres long and a few metres in diameter. As a consequence of ozone association followed by sodium oxide reduction in the presence of atomic oxygen, production of excited Na atoms in the (<sup>2</sup>P) state occurs followed by emission of the well known D lines. On a simple model ground state Na (<sup>2</sup>S) atoms are continually recycled acting as a catalytic agent. A meteor of fireball magnitude should deposit sufficient sodium to produce a visual enduring train<sup>5</sup>. It is possible, however, that under certain circumstances removal of Na atoms may occur, a process being permanent loss by charge exchange. Although the night-time concentrations of atmospheric positive ions in the upper D region are too low to be effective, meteoric metal ions present in the diffusing meteor column may be significant in transferring charge to free Na atoms



where  $M^+$  represents metal ions ( $M = Fe, Mg, Si$ ). The most abundant meteoric ions  $O^+$  are rapidly lost by charge exchange with the major atmospheric neutrals rather than by equation (1). This reaction represents a permanent loss of Na as radiative recombination or a sequence of reactions involving the major atmospheric neutrals results in negligible re-emergence of Na atoms. Metal ions themselves can suffer loss by association with atmospheric neutrals the most important route at 85 km is



giving metal oxide ions which dissociatively recombine with

electrons rapidly and at much faster rate than reduction by atmospheric O to restore  $M^+$ .

There are two significant factors in determining the spatial distribution of species in the expanding train. First, because of the relatively large extent of their coulomb fields, positive ions possess a smaller momentum transfer mean free path than neutral atoms, the radius of the cylindrical ion column at train formation is expected to be less than that of the neutral atom column. Second, the diffusion coefficient of free sodium atoms in atmospheric gases is several times greater than that of positive meteoric ions (which diffuse at the ambipolar rate). Consequently as the atomic Na and  $M^+$  columns have different radii throughout their expansions a proportionately greater loss rate of Na near the column axis due to equation (1) results. Because Na atoms are mopped-up much faster in the central regions of the meteor train a trough may develop in which relatively few Na ( $^2P$ ) atoms are produced. In addition, by equation (2) ozone molecules are depleted<sup>7</sup> much faster in the central regions of the column, so that fewer  $O_3$  molecules are available to take part in the catalytic cycle with Na which leads to the production of Na ( $^2P$ ). There are, therefore, two processes leading to a relatively low concentration of excited sodium atoms in the central regions of an expanding train. The depth and lifetime of the trough is determined by the speed of the chemistry, equations (1) and (2), compared with the effects of diffusion in restoring the equilibrium radial profile by transporting Na and  $O_3$  inwards and eliminating the hollow. It is also significant that the central hollow will only develop if the total number of  $M^+$  ions in a cross section of the train,  $\alpha_{M^+}$ , greatly exceeds the number of Na atoms,  $\alpha_{Na}$ . As the ionisation probability,  $\beta$ , for ionising collisions between meteoric atoms and atmospheric neutrals increases rapidly with increasing meteoroid velocity, the hollow meteor train is expected to be a high velocity phenomenon.

Early in the life of a train when molecular rather than eddy diffusion governs the radial expansion, the loss of catalytic sodium may be described essentially by the equations:

$$\frac{\partial}{\partial t} [Na] = D_{Na} \nabla^2 [Na] - \Sigma k_i [Na] [M^+]$$

$$\frac{\partial}{\partial t} [M^+] = D_a \nabla^2 [M^+] - k_1 [Na] [M^+] - k_{2M^+} [O_3] [M^+]$$

$$\frac{\partial}{\partial t} [O_3] = D_{O_3} \nabla^2 [O_3] - \Sigma k_{2M^+} [O_3] [M^+]$$

where  $D$  is the appropriate diffusion coefficient,  $\nabla^2$  the Laplacian in cylindrical coordinates and the number densities of various species are represented by their appropriate species symbol. Numerical solutions were obtained for a height of 85 km taking night-time  $[O_3] = 8 \times 10^8 \text{ cm}^{-3}$ . The adopted rate coefficients ( $\text{cm}^3 \text{ s}^{-1}$ ) are (ref. 8)  $k_1 = 2.4 \times 10^{-9}$  (independent of the positive ion),  $k_{2Fe^+} = 1.5 \times 10^{-10}$ ,  $k_{2Mg^+} = 2.3 \times 10^{-10}$  and  $k_{2Si^+} = 1.0 \times 10^{-10}$  (estimate). The diffusion coefficient ( $\text{cm}^2 \text{ s}^{-1}$ ) of sodium atoms in air is a factor of  $\sim 3$  greater than ambipolar<sup>9</sup> and at 85 km with  $T = 185 \text{ K}$  we adopt  $D_{Na} = 4.2 \times 10^4$  and  $D_a = 1.4 \times 10^4$ . A value of  $D_{O_3} = 4 \times 10^4$  is assumed. It is further assumed that following the ablation process, a meteor column is established whose constituent species have gaussian radial distributions described by radii  $r_i$  such that  $r_i^2 \propto D$ . For a meteor of fireball magnitude we take  $r_1$  (Na) = 20m. The results of Sida<sup>10</sup> are used for a working model giving  $\beta$  for meteoric atoms as a function of velocity while atom abundances appropriate to chondrite meteorites are adopted. Visual meteor magnitude, meteoroid pre-entry mass and velocity may be related<sup>11,12</sup> the line densities,  $\alpha$ , of deposited atoms and ions. The ratios  $\alpha_{M^+}/\alpha_{Na}$  for 30  $\text{km s}^{-1}$  and 60  $\text{km s}^{-1}$  are 0.26 and 3.9 respectively. The sodium D line emission per unit volume is equal to the rate of production of Na ( $^2P$ ) atoms so that for an optically thin column the apparent surface brightness (luminance) of any part of the train is proportional to the integral of the

product of the Na and  $O_3$  concentrations in a column of unit area along the line of sight. Because  $\beta$  is highly velocity dependent, significant Na loss occurs only in trains of high velocity meteors. A central hollow only develops for  $v > 50 \text{ km s}^{-1}$ . Solutions are shown in Fig. 1 where the peak to trough luminance ratio is about 2 at 60  $\text{km s}^{-1}$  while at 70  $\text{km s}^{-1}$  is about 3.5. These ratios result from severe depletion of Na ( $^2P$ ) atoms within a cylinder of radius about 20 m. A hollow train 60s after formation would be resolved by the naked eye at a distance of about 150 km. Such characteristics are in accord with observations. Although hollow train observations are sparse, the cases reported by Newton<sup>1</sup> refer exclusively to members of the Leonid shower (72  $\text{km s}^{-1}$ ) while those by Trowbridge<sup>2</sup> occurred at the epochs of the Orionids (66  $\text{km s}^{-1}$ ) and Perseids (60  $\text{km s}^{-1}$ ). The phenomenon has not been reported for the low velocity streams such as the abundant Geminids (36) Jacobinids (23) and Quadrantids (40  $\text{km s}^{-1}$ ).

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1. Newton, H. A. *Am. J. Sci. Arts* **47**, 399–413 (1869).
2. Trowbridge, C. C. *Astrophys. J.* **26**, 95–116 (1907).
3. Hughes, D. W. *Nature* **257**, 533–534 (1975).
4. Baggailey, W. J. *Nature* **257**, 567–568 (1975).
5. Baggailey, W. J. *Nature* **267**, 376 (1977).
6. Millman, P. M. in *From Plasma to Planet*, Nobel Symposium 21 (ed. ELvius, A.) 157–166 (Interscience, New York, 1972).
7. Poole, L. M. G. & Nicholson, T. F. *Planet. Space Sci.* **23**, 1261–1277 (1975).
8. Brown, T. L. *Chem. Rev.* **73**, 645–667 (1973).
9. Mitra, V. *Ann. Geophys.* **29**, 341–351 (1973).
10. Sida, D. W. *Mon. R. astr. Soc.* **143**, 37–47 (1969).
11. Verniani, F. *Space Sci. Rev.* **10**, 230–261 (1969).
12. Gadsden, M. *Tables of Meteor Ablation* ESSA Tech. Rep. IER 42-ITSA 42 (Institute for Aeronomy, Boulder, Colorado, 1967).

## Surface temperature of early Earth

CHANGES in the surface temperature of the Earth throughout its history are important for understanding both the geological development of the Earth's surface and the origin and development of life on Earth. The calculation of variations in the surface temperature with time is complicated by the number and nature of the physical variables involved, as well as by the long period of time over which extrapolation is required. Theoretical studies must be carried out in terms of highly simplified global atmospheric models with fairly lax boundary conditions. Consequently, even the gross features of atmospheric evolution may be explicable in terms of more than one theoretical model. Here we take the model most widely referred to in recent years and point out that a radically different approach can provide an equally supportable result. Too much significance should not, therefore, be read into the details of currently constructable models.

Sagan and Mullen<sup>1</sup> carried out a theoretical investigation of long-term changes in the Earth's surface temperature on the assumption that the major infrared absorbing gases in the Earth's atmosphere have always been water vapour and carbon dioxide. But, in view of the accepted boundary conditions for the early Earth, they concluded that the original terrestrial atmosphere must also have contained additional absorbing gases. This conclusion has two important consequences. First, the Earth's early atmosphere must have undergone a significant change in chemical composition as the postulated additional absorbing agency was removed from circulation. Second, because any physically probable additional absorber is likely to belong to a chemical species that figures in current discussions concerning the origin of life (Sagan and Mullen consider ammonia to be the most probable candidate) acceptance of their model has implications for ideas on the early development of life on Earth. We intend to demonstrate here that the same boundary conditions can be satisfied by a different model that postulates absorption by water vapour and carbon dioxide only throughout the lifetime of the Earth.

In principle, the surface temperature at any epoch can be

calculated in two stages. The first involves the computation of the effective temperature of the planet ( $T_e$ ) by the equation

$$S(1 - A) = f\sigma T_e^4 \quad (1)$$

where  $S$  is the solar constant;  $A$  the spherical albedo of the Earth;  $f$  the flux factor;  $\sigma$  the Stefan-Boltzmann constant. All the factors are straightforward except for  $f$ , which we will now define. For a rapidly rotating planet with a thick atmosphere, the area emitting radiation is taken to be  $4\pi R^2$  (where  $R$  is the planetary radius). For a slowly rotating planet with a thin atmosphere, the corresponding emitting area is  $2\pi R^2$ . Since the area receiving solar radiation is  $\pi R^2$ , the flux factor (defined as the ratio of the emitting area to the absorbing area) must, therefore, be either 4 or 2. But the choice need not always be so obvious. In physical terms, the value assigned to the flux factor depends on the ratio of two characteristic times. The first ( $\tau_h$ ) is the time required for a planetary atmosphere to radiate away a major part of its heat content: the second is the rotational period of the planet ( $\tau_r$ ). If  $\tau_h/\tau_r \approx 1$ , neither  $f = 4$ , nor  $f = 2$ , represent good approximations, and it becomes necessary to use a transitional value (for a more detailed discussion of  $f$  see ref. 2).

The second stage of the calculation relates  $T_e$  to the surface temperature ( $T_s$ ) by an equation of the general form

$$T_s = T_e + \Delta T \quad (2)$$

(Here  $\Delta T$  is the 'greenhouse' increment due to the presence of an atmosphere, and depends on the mass, surface pressure and chemical composition of the atmosphere concerned.) We have, therefore, set up a computer program that solves equations (1) and (2) for  $T_s$  over a wide range of the relevant input parameters and at any epoch. Our approach is similar to that of Sagan and Mullen, though we have incorporated a reanalysis of the laboratory data available on infrared absorption as a function of pressure (for further details, see refs 2 and 3).

A determination of changes in the terrestrial surface temperature entails a study of all possible secular variations in the input parameters over the lifetime of the Earth (taken to be  $4.5\text{--}4.6 \times 10^3$  Myr). The best attested change is in the solar constant. There is good agreement between theoretical models of the Sun, showing that  $S$  has increased by 40–45% since the origin of the Solar System<sup>4</sup>. If this conclusion is fed into the model computations for  $T_s$ , then curve Fig. 1*b* results, giving a surface temperature well below the freezing point of water during the early part of the Earth's history. There is, however, an important observational limitation on the minimum surface temperature of the early Earth. Geological evidence suggests the presence of extended sheets of liquid water on the Earth's surface at least 3,700 Myr ago<sup>5</sup>. We cannot use this as an index of the degassing rate, as the actual volume of water cannot be readily determined,

but we can assert that the average surface temperature could not at that time have been much below 273 K.

Sagan and Mullen escape from this dilemma by suggesting that the early terrestrial atmosphere contained an additional absorbing agency. They thus increase  $\Delta T$  until the required minimum value of  $T_s$  is reached. But the same final value of  $T_s$  might be obtained by increasing  $T_e$ . If a smaller value of  $S$  is accepted, then it can be compensated, according to equation (1), by making  $A$  larger,  $f$  smaller, or by changing both simultaneously. We investigate here whether such changes can provide an acceptable alternative model.

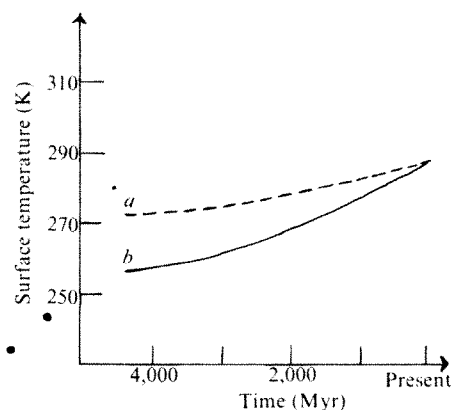
Sagan and Mullen assumed that the terrestrial albedo has always been approximately the same; but this assumption depends on the precise mode of formation of the atmosphere. It is generally supposed that the Earth's atmosphere (together with the hydrosphere and some of the sedimentary material) originated by degassing from the crust. If the atmospheric gases were released over an extended period of time, we would expect the albedo to change with time—from an initial value appropriate to an atmosphereless planet (say, 0.07) to the present-day value of 0.33. We have computed the run of the surface temperatures for a model where the gases forming the terrestrial atmosphere have been vented throughout the planet's existence. The terrestrial albedo has been taken to be a function of the mass of atmospheric gases per unit area. This approximation depends on the assumption that the planetary albedo is primarily affected by the presence of clouds, which can, to some extent, be correlated with atmospheric mass.

The results are shown in Fig. 1*a*. A comparison of the two curves in Fig. 1 indicates that the variation in albedo partially compensates for the change in the solar constant. Consequently, a relatively slow degassing leads to an average surface temperature that never falls far below the freezing point of water.

So far we have considered changes in the albedo due to continuous degassing. Available K/Ar data suggest that degassing went on more rapidly earlier in the Earth's history than now, though uncertainties (such as the supply of potassium in the crust) make it difficult to attach a figure to this difference<sup>6</sup>. On our present argument, a more rapid early degassing would imply a lower average surface temperature. But, there remains one further parameter in equation (1) that requires examination—the flux factor,  $f$ . As with the albedo, the choice of a value for the flux factor in atmospheric models has been considered straightforward, and for the present-day Earth, there is no ambiguity over the flux factor to be used:  $\tau_h/\tau_r \approx 10^2$ , and  $f = 4$ . But the rotational period of the Earth is believed to have varied with time, owing to tidal interaction with the Moon, increasing from an original value of somewhat more than 2 h to the current figure of 24 h. If the Earth's atmosphere had undergone no major change with time, this rotational variation would clearly not affect the appropriate value of  $f$ . If, however, the terrestrial atmosphere has built up by degassing during the Earth's existence, then the value of  $f$  throughout the early period requires re-examination. It is generally supposed that the Moon was either captured, or formed in orbit, not later than 4,000 Myr ago. (Later capture should have led to observable effects in the geological record.) This implies that the major tidal interaction between the Earth and the Moon (and, therefore, the most rapid deceleration of the terrestrial rotation) occurred at the same time as our postulated initial degassing. Hence, both  $\tau_h$  and  $\tau_r$  would be changing simultaneously.

We have recomputed our values for the surface temperature of the early Earth taking into account concurrent changes in the value of  $f$ . (The rate of change of the Earth's rotation has been taken from Marsden and Cameron<sup>7</sup>.) The results are shown in Fig. 2. The significant consequence is that the average surface temperature of the early Earth is appreciably enhanced. This implies that changes in the albedo and flux factor can be set against each other to produce a range of possible temperatures for the early Earth. More particularly, the effects of an enhanced early degassing rate can be cancelled by an appropriately varying flux factor. Figure 2 clearly suggests that the early Earth may even

Fig. 1 Increase in the surface temperature of the Earth with increase in solar luminosity: *a*, for varying albedo; *b*, for constant albedo.





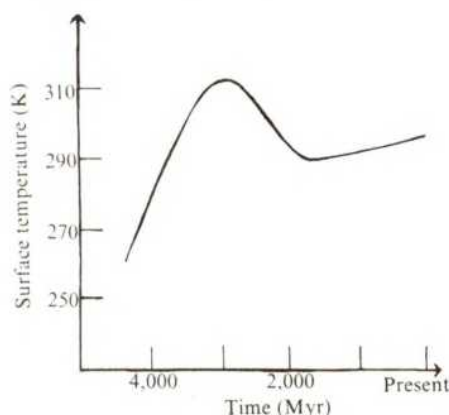


Fig. 2 Possible evolution of surface temperature for the Earth allowing for variations in the flux factor. These are caused by changes in terrestrial rotation due to the capture of the Moon.

have been warmer than the present-day Earth. It is, therefore, worth pointing out that some recent palaeotemperature measurements support this possibility<sup>8</sup>.

The model presented here is capable of fulfilling the boundary conditions without requiring the presence of additional absorbing agencies. More generally, we would argue that current atmospheric evolution models are too primitive to provide certain theoretical backing for an early reducing atmosphere on Earth. Hence, they must be used with great caution in discussions concerning the origin of life.

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1. Sagan, C. & Mullen, G. *Science* **177**, 52 (1972).
2. Henderson-Sellers, A. thesis, Univ. Leicester (1976).
3. Henderson-Sellers, A. & Meadows, A. J. *Planet. Space Sci.* **24**, 41 (1976).
4. Haselgrove, C. B. & Hoyle, F. *Mon. Not. R. astr. Soc.* **119**, 112 (1959).
5. Schildowski, M., Eichman, R. & Junge, C. E. *Precambrian Res.* **2**, 1 (1975).
6. Fisher, D. E. *Nature* **256**, 113-4 (1975).
7. Marsden, B. G. & Cameron, A. G. W. (eds) *The Earth-Moon System* (Plenum, New York, 1966).
8. Knauth, L. P. & Epstein, S. *Geochim. cosmochim. Acta* **40**, 1095 (1976).

## Redox regulation of atmospheric oxygen and its consequences

MANY attempts<sup>1-9</sup> have been made to explain why the concentration of atmospheric oxygen remains steady at ~21%. I previously suggested that the turnover of marine and brackish water sediments promotes a recurrent demand for dissolved oxygen by alternately oxidising and reducing insoluble inorganic iron. Oxygen incorporated with sediments by this means combines with hydrogen to form water—thus preventing to some extent that loss of hydrogen by which Earth is oxidised<sup>4,7</sup>. It is now apparent that regulation of molecular oxygen cannot be brought about by iron alone because the rate of turnover (even of shallow water sediments) is not fast enough<sup>13</sup>. Nor can sulphur (on its own or in association with iron) effect the transfer of sufficient oxygen from the aerobic to the anaerobic environment if rates of microbial activity in deep sea sediments are generally 1-2 orders of magnitude slower than in those of the continental shelves<sup>12,15</sup>. I propose, therefore, that six elements (N, Mn, Fe, S, C, H) circulate in reduced and oxidised forms between the aerobic and anaerobic environments and bring about 'redox regulation of atmospheric oxygen'.

Entering the anaerobic environment as the oxidants  $\text{NO}_3^-$ ,  $\text{MnO}_2$ ,  $\text{FeOOH}$ ,  $\text{SO}_4^{2-}$ ,  $\text{CO}_2$  and  $\text{OH}^-$ , N, Mn, Fe, S, C and H

are reduced to  $\text{N}_2$ ,  $\text{Mn(II)}$ ,  $\text{Fe(II)}$ ,  $\text{H}_2\text{S}$ ,  $\text{CH}_4$  and  $\text{H}_2$  at successively lower levels of redox potential—the reductions taking place in terrestrial soils, marine and freshwater sediments and the guts of animals. To complete their cycles (satisfying their oxygen demands as reductants)  $\text{N}_2$ ,  $\text{Mn(II)}$ ,  $\text{Fe(II)}$ ,  $\text{H}_2\text{S}$ ,  $\text{CH}_4$  and  $\text{H}_2$  must be freed from the anaerobic environment. This happens by venting:  $\text{N}_2\uparrow$ ,  $\text{CH}_4\uparrow$  and  $\text{H}_2\uparrow$ , by diffusion:  $\text{H}_2\text{S}$ , and by turnover:  $\text{Mn(II)}$  and  $\text{Fe(II)}$ . The inorganic oxygen demands of  $\text{Mn(II)}$ ,  $\text{Fe(II)}$  and  $\text{H}_2\text{S}$  are then satisfied by dissolved oxygen, whilst those of  $\text{N}_2$ ,  $\text{CH}_4$  and  $\text{H}_2$  are satisfied by reactive forms of gaseous oxygen ( $[\text{O}]$ ,  $\text{O}_2$ ,  $\text{O}_3$ ). On this basis all of them make recurrent demands on molecular oxygen though the demands of N, S and C (which circulate in vast quantities, combine with more than their own weights of oxygen and have their reductions brought about by microorganisms using dead organic matter as the reductant) have the greatest effect.

Redox cycling poises the aerobic environment at  $pE \sim 13$  on an anaerobic environment extending from a level at which  $\text{NO}_3^-$  is reduced to gaseous nitrogen ( $pE \sim 12$ ) to that at which water yields molecular hydrogen ( $pE \sim -7$ ). ( $pE \text{ (RTF}^{-1} \ln 10) = Eh$  where  $Eh$  is the electrode potential of a half-cell measured against the standard hydrogen half-cell which by convention has a potential of 0V). In terms of  $pE$ , it is significant that regulation was achieved shortly after N became an oxygen sink, and that it is currently held at a level below that at which widespread forest and grassland fires would be limiting<sup>4</sup>. It is suggested that the 'gate' between the aerobic and anaerobic environments is 'guarded' by

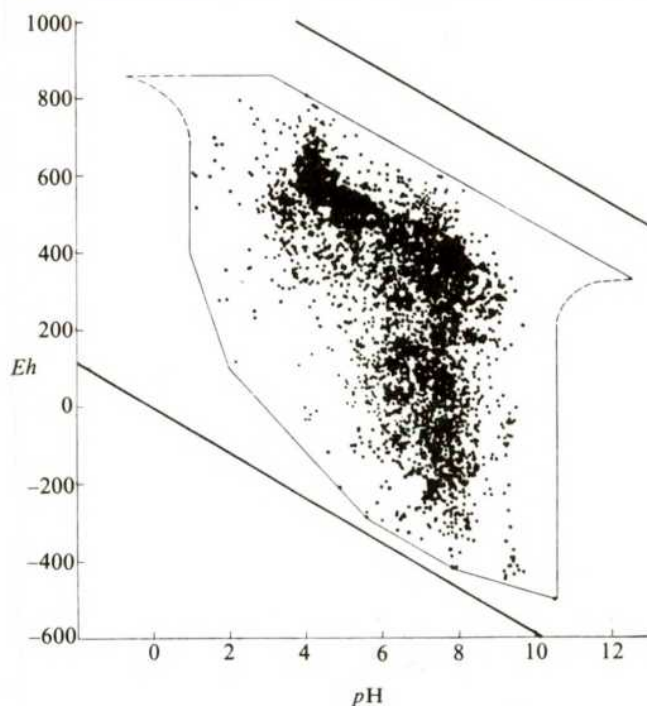


Fig. 1 Distribution of  $Eh$ - $pH$  measurements of natural aqueous environments (after Baas Becking *et al.*<sup>10</sup>).

the processes of nitrogen fixation and denitrification. The former, using more molecules of oxygen than molecules of nitrogen, tends to lower oxidation potential to a point where the way is opened for denitrification. Such a lowering of  $pE$  can be viewed variously: as a displacement of one redox reaction by the one beneath it in the series, as a general rise of these reactions within a soil or sediment profile, as a slight telescoping of the anaerobic environment at the aerobic end. If nitrifying bacteria at the surface of a sediment or soil are inactivated by a lowering of oxidation potential, denitrifiers alongside them are activated and use the  $\text{NO}_3^-$  made elsewhere. Thus, normally, and in concert with the other cycles, the highly  $pE$ -sensitive N cycle is able to correct any tendency to over-regulation, so that despite the favourable  $pE$  and

pH of Earth's surface, an excess of  $\text{NO}_3^-$  (refs 5, 7, 11, 14) is as unlikely as a shortage of oxygen.

Functioning as a sink for oxidants, the reducing anaerobic environment helps to regulate the pH of the aerobic. This is evident in Fig. 1—a plot of some 6,000 Eh–pH readings (including those of other workers) made by Baas Becking *et al.*—which shows a marked columnar disposition of points between pH 7–8 in the anaerobic, surmounted in the aerobic by a linear concentration having the same slope ( $-59\text{mV}$  per pH unit) as the equilibria between water and oxygen and water and hydrogen. It is apparent that the reducing, anaerobic environment maintains a state of near neutrality throughout its range whilst the aerobic environment becomes more acid as it becomes more oxidised.

In the aerobic environment, this tendency to acidity as oxidants go into solution is offset by carbonate and aluminosilicate<sup>7</sup> buffering. There are many places, however, where buffers are lacking and increases in acidity happen either because of a seasonal hiatus in the uptake and eventual reduction of oxidants (as in Norway where they are retained through winter in the snow) or because the anaerobic environment is too poorly developed to cope with the influx.

There are other places where the anaerobic environment is atypical and there are anoxic basins, in which oxidants are in short supply, and deserts, from which water and the reductants furnished by dead organic matter are missing. Earth is adjusted to their presence and it may be wrong to suppose that they are signs of environmental ill health. Nevertheless, overall, Earth's aerobic and anaerobic environments are mutually sustaining; to diminish one is to diminish the other; to have failed to acquire one would have meant failing to acquire both.

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Received 23 June; accepted 8 October 1977.

1. Berkner, L. V. & Marshall, L. C. *J. Atmos. Sci.* **22**, 225 (1965).
2. Board, P. A. *Atmos. Envir.* **10**, 339 (1976).
3. Garrels, R. M. *et al. Am. Sci.* **64**, 306 (1976).
4. Lovelock, J. E. & Lodge, J. P. *Atmos. Envir.* **6**, 575 (1972).
5. Lovelock, J. E. & Margulis, L. *Tellus* **26**, 1–2 (1974).
6. Sillen, L. G. *Acta. Chem. Scand.* **18**, 1016 (1954).
7. Sillen, L. G. *Tellus* **18**, 198 (1966).
8. Van Valen, L. *Science* **171**, 439 (1971).
9. Walker, J. C. G. *Am. J. Sci.* **274**, 193 (1974).
10. Baas Becking, L. G. M. *et al. J. Geol.* **68**, 243 (1960).
11. Hutchinson, G. E. in *The Solar System* (ed. Kuiper) (University of Chicago Press, Chicago, 1954).
12. Jannasch, H. W. & Wirsén, C. O. *Science* **180**, 64 (1973).
13. Jørgensen, B. B. *Limnol. Oceanogr.* (in the press).
14. Lewis, G. N. & Randall, M. in *Thermodynamics and the free energy of chemical substrates* (McGraw-Hill, London, 1923).
15. Smith, K. L. & Teal, J. M. *Science* **179**, 282 (1973).

## Has man increased stratospheric ozone?

MANY anthropogenic threats to the ozone layer have surfaced (eight so far) since McDonald first suggested that supersonic transport aircraft would lead to increased skin cancer. It should be comforting, therefore, that agricultural modifications to the Earth (increased oxygenation, drainage, and pH of the soil and destruction of bacteria culturing humus) are suggested here to be more important than addition of fixed nitrogen in modifying  $\text{N}_2\text{O}$  production by denitrifying bacteria. If true, man should have caused an increase in total global ozone.

This effect operates through the denitrification branch of the nitrogen cycle in which soil bacteria, in the absence of oxygen, turn to nitrate and nitrite as oxygen donors and produce  $\text{N}_2$  and  $\text{N}_2\text{O}$  as waste byproducts which escape into the atmosphere. Part of the  $\text{N}_2\text{O}$  ascends by the Hadley circulation into the stratosphere where a fraction, estimated at about 1% of the total surface production, is oxidised to produce the catalytic destroyer of ozone— $\text{NO}$ . While most of this cycle is known only qualitatively, it is believed that denitrification is favoured in soils which: (1) are saturated with water, (2) are largely devoid of oxygen, (3) contain

organic matter for microbial consumption, and (4) contain oxidised nitrogen in the form of nitrate or nitrite. (The latter has been the only factor considered in most discussions of this problem<sup>1</sup>.) According to Johnston<sup>2</sup>, the fraction of denitrified nitrogen released to the atmosphere as  $\text{N}_2\text{O}$  is favoured by: (1) low temperature, (2) low pH, and (3) marginal anaerobic conditions; all of which in turn lead to a low rate of denitrification. Thus the net effect of these last three parameters on  $\text{N}_2\text{O}$  production was left ambiguous. Most authors seem to feel they cause a net reduction in the rate of  $\text{N}_2\text{O}$  production<sup>3</sup>.

Since taking up agriculture, man's tillage of the soil and elimination of natural standing water have had large scale effects.

First, soil oxygen has been increased through: (1) loosening and turning, producing increased aeration and (2) reduced competitive oxygen demand from root growth by: spacing of monocultured plants; elimination of competing plants or weeds, and fallowing; (3) reduction of oxygen-excluding soil moisture through: increased runoff; erosion and oxidation of water-retaining humus and non-return of plant debris; measures to improve drainage and/or percolation wherever water stagnation restricted plant growth.

Second, the volume of culture medium for soil bacteria has been reduced through the loss of humus and top soil.

There have, of course, been opposing effects favouring increased  $\text{N}_2\text{O}$  production from irrigation, rice culture and addition of fixed nitrogen to the soil but these are generally on a smaller scale, and tend to occur under conditions or in areas where they have little or no effect. For example, irrigation and to a lesser extent fertilisation is restricted to porous and well drained soils, large scale fertilisation has turned from nitrate to ammonia (oxidation of ammonia to nitrate will occur but only under conditions at least temporarily unfavourable for denitrification) and rice culture is restricted to areas already tending to be waterlogged. There have been numerous claims that anthropogenetically produced oxides of sulphur and nitrogen have caused large scale increases in the acidity of precipitation and a suggestion<sup>4</sup> that this could lead to increased production of  $\text{N}_2\text{O}$ . This is a logical theoretical extrapolation but it has little support from available data. As noted above, by slowing denitrification a lower pH may actually reduce  $\text{N}_2\text{O}$  production. Furthermore, there are large natural injections into the atmosphere of fixed gaseous sulphur and nitrogen exceeding those due to man. Without the return to the soil by the atmosphere of these soluble plant nutrients we could expect large biological deserts even where there is adequate rainfall. The areas in which decreased pH has been claimed represent only a small portion of the globe and past data to establish the trend are very fragmentary.

It thus seems plausible that any affect that man has had or will have in the foreseeable future on denitrification is to decrease  $\text{N}_2\text{O}$  production and thus to increase the depth of our ultraviolet-screen of stratospheric ozone. Available data on tropospheric concentrations of  $\text{N}_2\text{O}$  are very confusing<sup>5</sup> and of little help in resolving this question. The data suggest abrupt increases in  $\text{N}_2\text{O}$  concentration between 1967 and 1968 and again in recent years. The difference, however, in  $\text{N}_2\text{O}$  concentration indicated by the different sets of measurements raises questions about the accuracy of the measurements. The ozone measurements are more encouraging. They suggest, that total global ozone has been increasing since measurements were first made in the early 1920s and particularly since the significant expansion in the observational network began about the mid-1950s.

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Received 17 August; accepted 28 October 1977.

1. Shapley, S. *Science* **195**, 658 (1977).
2. Johnston, H. S. *Science* **195**, 1280 (1977); *J. Geophys. Res.* **82**, 1711 (1977).

3. Russell, E. W. in *Soil Conditions and Plant Growth* 10th edn (Longman, New York, 1973).  
 4. McElroy, M. B. *Int. Rev. Sci.* **9**, 127-211 (1976).  
 5. Pierotti, D. & Rasmussen, R. A. *Jt Symp. Non-Urban Tropospheric Composition*, Hollywood (1976).

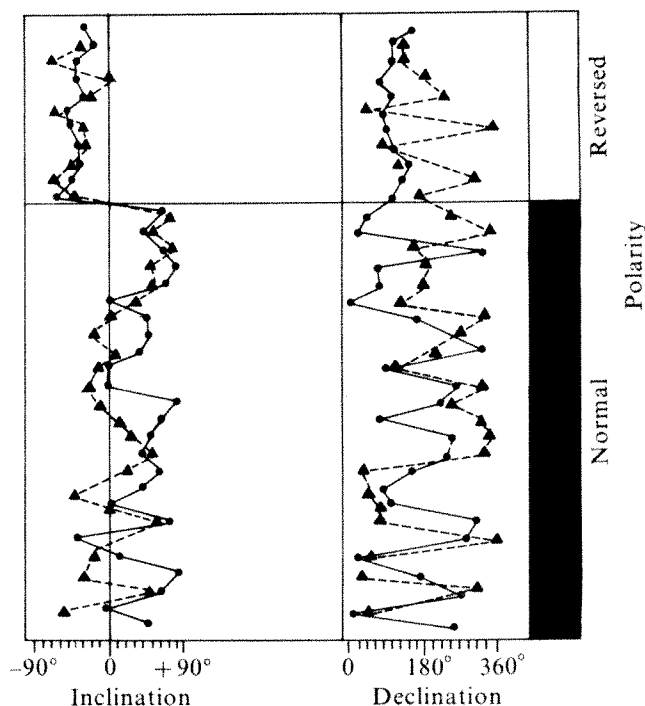
## Evidence of rapid changes in the Permian geomagnetic field during the Zechstein marine transgression

CONSIDERABLE refinements have been made recently to Palaeozoic magnetic reversal stratigraphy. Here, we present evidence of a previously unrecognised rapid field change of positive polarity during the Upper part of the Kiaman Magnetic Interval. This is recorded in the Upper Permian Marl Slate, a well-known stratigraphic marker horizon and should be of considerable value to future palaeomagnetic and stratigraphic studies.

Late Palaeozoic palaeomagnetic data are voluminous, largely because of the ubiquity of easily measured red beds and igneous rocks. Within these data there are systematic discrepancies and it has been suggested that the Permian geomagnetic field did not perfectly correspond to an axial dipole. Palaeontological diversity data have also been used to argue that the Permian field was non-dipole<sup>1</sup>. A number of palaeomagnetists have noted that pole positions plotted with respect to the Bullard *et al.*<sup>2</sup> reconstruction are, for the southern continents, displaced by about 20° to the east, although this has been explained in terms of an alternative reconstruction<sup>3</sup>. Briden *et al.*<sup>1</sup> investigated Permian and Triassic palaeomagnetic data in detail and were able to show that, at a first approximation, the Permian field corresponds to an axial dipole when based on a Bullard-type fit. One of the important discrepancies in this model is that for European data, inclinations are often much less than expected. These were interpreted in terms of coaxial multipole fields and our results provide further evidence of short lived anomalies in the Permian geomagnetic field. We still believe, however, that the best overall model of the Permian field is that of a geocentric axial dipole.

An important feature of the Permian geomagnetic field is that it was persistently of reversed polarity. From late Carboniferous to late Permian times, the geomagnetic field was predominantly reversed and this has been called the Kiaman Magnetic Interval. One brief normal event occurring at approximately the Carboniferous-Permian boundary has been documented in the USSR and the USA<sup>4</sup>. Here we record the discovery of further rapid field changes of positive polarity occurring at a stratigraphically significant horizon which could be of considerable value to studies of Permian strata.

Our results come from an investigation of the role of metal sulphides in palaeomagnetism and, specifically, from the Upper Permian Marl Slate. This is a thin, black, sapropelic, dolomitic laminite which represents the Zechstein marine transgression over Rotliegendes sandstones in northwestern Europe<sup>5</sup>. The eastern European equivalent of the Marl Slate is the German Kupferschiefer. A complete sequence of the Marl Slate has been obtained from a borehole drilled off the north-east coast of England by the National Coal Board at NZ5099852007 and donated for study by Dr D. Magraw. In this borehole, the Marl Slate was cored from a depth of 285.3 m and is 1.18 m thick. Thirty-six cores of 2.5-cm diameter were drilled parallel to the bedding and throughout the length of the core. In this way, palaeomagnetic directions could easily be referred to the bedding planes and true palaeolatitudes calculated. Only the relative declination could be measured, because the absolute orientation of the core relative to geographic North is unknown. The natural remanent magnetisation (NRM) of the specimens was measured along with their low-field susceptibility. The results are summarised in Fig. 1. The NRM intensity of the Marl Slate is weak, of the order of 0.1  $\mu\text{G}$  and the initial susceptibility varies between 5 and



**Fig. 1** Magnetic stratigraphy of the Marl Slate showing an upper zone of negative polarity and a lower zone of positive polarity. The section is 1.18 m thick. Declination is relative, inclination is absolute. The results are shown before heating (●) and after heating (▲).

14  $\mu\text{G Oe}^{-1}$ . The initial directions fall into two well defined groups: an upper zone with negative inclinations, and a lower zone of positive inclinations. The mean direction of magnetisation is dec. 299°, inc. +34°,  $\alpha_{95}$ .19°, after the polarity of the upper group has been normalised. In these initial measurements, there are important differences in the degree of scatter of both inclination and declination between the positive and negative zones. In the upper, negative part of the core, both declination and inclination show much less systematic variation than the lower, positive part of the core (Fig. 1). The transition from positive to negative inclination is extremely sharp but there is no sharp transition in declination, although this may be due to the fact that the directions have relatively steep inclination, and would not be expected to show such a sharp transition.

Thermal demagnetisation studies investigated the stability of the magnetisation and showed that the magnetisation of the Marl Slate in this core is extremely soft and only about 20% of the original remanence remains after heating to 200 °C. At and above this temperature, there are large increases in intensity due to a laboratory magnetisation caused by the oxidation of sulphide minerals<sup>7</sup>. During thermal demagnetisation, no recognisable secondary components were removed and after cleaning the specimens at 150 °C, the positive and negative zones in the core remained unaffected. There was little change in the mean direction of magnetisation of the core: dec. 324°, inc. +21°,  $\alpha_{95}$ .34°, although there was some increase in scatter, particularly in the declination. This results from the difficulty of resolving accurately the magnetisation of the specimens at much weaker NRM intensities (average 0.05  $\mu\text{G}$ ). For these reasons, we believe the initial measurements represent most accurately the original magnetisation of the Marl Slate.

Several factors suggest that the magnetisation of the Marl Slate in this core was acquired during deposition or early diagenesis. Because the core came from a submarine borehole, the specimens should be free from surface oxidation and this has been confirmed by detailed mineralogical investigations.



The presence of iron, zinc, copper and lead sulphides, of early diagenetic origin and showing no signs of subsequent alteration is taken as evidence that the original magnetic mineralogy is largely unchanged. Moreover, textural evidence indicates that the main mineral phase in the Marl Slate, dolomite, is also of early diagenetic origin. It is interlaminated with calcite and consists of thin laminae of dolomicrite and hypidiotopic dolomite<sup>8</sup>, features which indicate an early diagenetic origin. The NRM carrier cannot as yet be identified precisely, although rock magnetic studies indicate that it might be a mixture of magnetite and haematite.

X-ray diffraction and geochemical studies indicate that the lower part of the core is richer in detrital quartz, whereas the upper part has a higher carbonate content. We take this to indicate that the sedimentation rate was relatively rapid in the lower part and much slower in the upper part of the core (corresponding approximately to the negatively magnetised zone).

We are therefore able to interpret the magnetisation of the Marl Slate. The upper part represents a negatively magnetised zone, acquired over a relatively long time and incorporating ambient geomagnetic field changes. The lower part is a zone of positive magnetisation, acquired more rapidly and in which ambient geomagnetic fluctuations have not been averaged out. Although the upper negative zone may represent a part of the Kiaman reversed polarity, we do not believe that the lower positive zone is a simple normal polarity zone, but rather evidence of rapid field changes similar to an excursion. We believe that the recognition of this positive rapid field change in the upper Kiaman is particularly significant because of its stratigraphic location. It coincides with strata which record the Zechstein marine transgression and which can be widely recognised over northwestern Europe. Further palaeomagnetic studies should provide a better understanding of the timing of this transgression and also of the Permian geomagnetic field.

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1. Stehli, F. G. *J. geophys. Res.* **75**, 3325 (1970).
2. Bullard, E., Everett, J. E. & Smith, A. G. *Phil. Trans. R. Soc. A* **258**, 41 (1965).
3. Van der Voo, R. & French, R. B. *Earth Sci. Rev.* **10**, 99 (1974).
4. Briden, J. C., Smith, A. G. & Sallomy, J. T. *Geophys. J. R. astr. Soc.* **23**, 101 (1970).
5. McElhinny, M. W. (Cambridge University Press, London, 1973).
6. Stewart, F. H. *US Geol. Surv. Prof. Pap.* **440-Y**, 1 (1963).
7. Turner, P. *Sedimentology* **22**, 563 (1975).
8. Friedman, G. M. *J. sediment. Petrol.* **35**, 643 (1965).

## Hilgardite and parahilgardite piezoelectric zeolite type phases

THE calcium chloroborate minerals hilgardite and parahilgardite are classic examples of point group symmetries *m* and *1* respectively<sup>1,2</sup>. They were found in the Choctow salt dome, Louisiana and are both piezoelectric. The chemical composition, originally given as  $\text{Ca}_8(\text{B}_5\text{O}_{11})_4\text{Cl}_4 \cdot 4\text{H}_2\text{O}$  for both minerals (see refs 1 and 2), has been revised to  $\text{Ca}_2(\text{B}_5\text{O}_8(\text{OH})_2\text{Cl})$  (ref. 3). Our structure determination indicates the chemical composition to be  $\text{Ca}_2\text{B}_5\text{O}_9\text{Cl} \cdot \text{H}_2\text{O}$  for both phases.

Hilgardite is monoclinic, space group *Aa*, with cell dimensions:  $a = 11.438(2)$ ,  $b = 11.318(2)$ ,  $c = 6.318(1)$  Å,  $\beta =$

$90.06(1)^\circ$ ;  $Z = 4$ . Parahilgardite is triclinic, space group *P1* with cell dimensions:  $a = 17.495(4)$ ,  $b = 6.487(1)$ ,  $c = 6.313(1)$  Å,  $\alpha = 60.77(1)$ ,  $\beta = 79.56(1)$ ,  $\gamma = 83.96(2)^\circ$ ;  $Z = 3$ . The crystal structures have been determined by the heavy atom method and refined by the method of least squares to *R*-factors of 0.018 and 0.028 for hilgardite and parahilgardite respectively, based on 1,475 and 4,432 reflections, measured on an automatic single crystal diffractometer.

The structure of hilgardite is a three-dimensional borate framework, whose building block is the anhydrous pentaborate polyanion,  $(\text{B}_5\text{O}_{12})^{9-}$ , consisting of three  $(\text{BO}_4)$ -tetrahedra and two  $(\text{BO}_3)$ -triangles. Because all of the terminal oxygen corners are shared, the chemical composition of the borate framework is  $(\text{B}_5\text{O}_9)^{3-}$ . The pentaborate polyanions form chains parallel to the *c*-axis by sharing tetrahedral corners with those belonging to adjacent polyanions (Fig. 1). Within each chain, corners of two borate tetrahedra are pointed along  $+a$  and  $+b$  directions, whereas corners of two borate triangles are pointed along  $-a$  and  $-b$  directions; these corners are shared with four adjacent chains (Fig. 2), such that tetrahedral corners of one chain are shared with triangular corners of the other. An open borate framework is created this way, which has channels (diameter  $\sim 5.2$  Å) parallel to the *a*- and *c*- axes. The water molecules and the chlorine atoms occur within the channels,

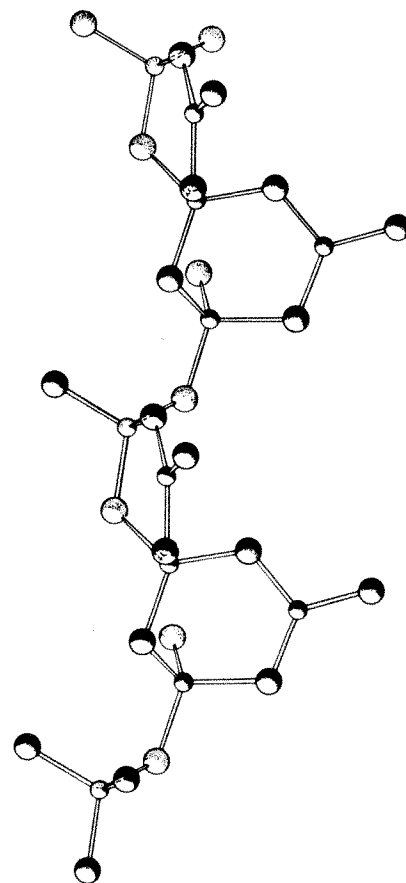
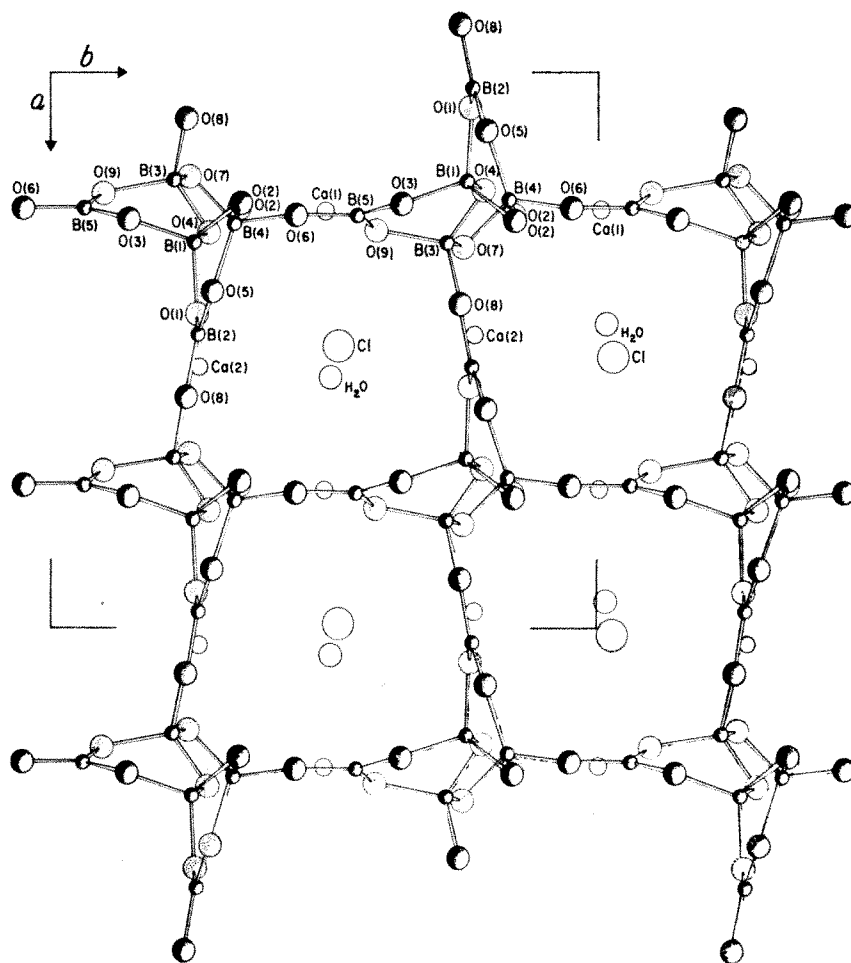


Fig. 1 The pentaborate polyanion chain in hilgardite parallel to the *c*-axis. A similar chain exists in parahilgardite.

whereas the calcium atoms occur at the edge of the channels running parallel to the *c*-axis. The crystal structure of parahilgardite is very similar to that of hilgardite, consisting of an open borate framework, formed of three crystallographically independent pentaborate units, where the calcium and chlorine atoms occur within open channels. Thus, hilgardite and parahilgardite are zeolite type phases. To our knowledge, this is the first report of borate zeolites. Ion-exchange experiments on these two minerals are in progress.

Fig. 2 The pentaborate framework structure of hilgardite projected down the *c*-axis, showing open channels (diameter 5.2 Å) parallel to *c*. Note that the chlorine atoms and water molecules occur within these channels.



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<sup>1</sup> Hurlbut, Jr, C. S. & Taylor, R. E. *Am. Miner.* **22**, 1052-1057 (1937).

<sup>2</sup> Hurlbut, Jr, C. S. *Am. Miner.* **23**, 765-771 (1938).

<sup>3</sup> Braitsch, O. *Beiträge Miner. Petrogr.* **6**, 233-247 (1959).

## Responses of a benthic marine invertebrate to $\gamma$ -irradiated sediment

THERE seems to be little information on the influence of high radiation levels on marine sediments and their subsequent recolonisation by sedimentary invertebrates. We report here that the benthic amphipod *Corophium volutator* (Pallas) responds to irradiated sediments in which there have been changes of *Eh* and microbial viability. Our results are of direct relevance to the microbiological and chemical properties of sediments<sup>1-3</sup>, and to the role played by microorganisms in habitat selection by benthic invertebrates<sup>4,5</sup>.

The first series of experiments (Table 1) demonstrates behavioural responses of *C. volutator* to irradiated compared with autoclaved and untreated sediments. Mud sediments were collected intertidally from the Clyde Estuary and wet-sieved through a 750- $\mu$ m sieve to remove large particles. Batches of sediment were

then divided into three parts. One part was the untreated control. The second was autoclaved at 121°C for 30 min and the third was exposed to  $\gamma$  irradiation for 18 h, giving a dose of 6 Mrad (cobalt source, Scottish Research and Reactor Centre, East Kilbride). Precautions were taken to ensure even irradiation and samples were immersed in a large water bath to prevent heating effects during irradiation.

Behavioural experiments were conducted with *C. volutator* in which animals were offered a choice of two sediments using standard techniques<sup>4</sup>. Animals preferred control to irradiated sediment and control to autoclaved sediment (Table 1). When autoclaved sediment was tested against irradiated, however, animals markedly preferred the autoclaved. This result was unexpected and led us to measure the physical and chemical properties of the autoclaved and irradiated sediments, to establish any differences between them. The most clearly defined difference was in redox potential. On replicate measurements the redox potential of the autoclaved sediment was -110 to -130 mV and of the irradiated sediment was -550 to -590 mV. Control sediments had an *Eh* of -50 to -65 mV.

We then investigated the relationship between increasing irradiation and *Eh*; heterotrophic bacterial counts were also taken (surface counts on Difco marine agar). The result (Fig. 1) shows that the heterotrophic bacteria decreased from  $2 \times 10^7$  cells per g dry weight in the unirradiated sediment to less than  $1 \times 10^2$  cells per g dry weight in the sediments irradiated with 0.5 Mrad or more. On the other hand *Eh* remained constant to 1.5 Mrad, and then declined to very low values of -500 to -600 mV at 4 to 6 Mrad. The difference in the shape of the two curves allowed us to select irradiation treatments of 0.8 Mrad and 5.4 Mrad which would give sediments of normal *Eh* (about -100 mV) and very low *Eh* (about -600 mV), respectively, while both would be virtually sterile (less than 10 cells per g). These two irradiation

**Table 1** Preferences of *C. volutator* for control, irradiated and autoclaved sediments

Expt	control sediment	% animals in autoclaved sediment	Total no. animals burrowed
(1)	66	34	85
Expt	control sediment	% animals in irradiated sediment	Total no. animals burrowed
(2)	78	22	87
Expt	autoclaved sediment	% animals in irradiated sediment	Total no. animals burrowed
(3)	71	29	45

Each experiment was run in triplicate using three choice dishes<sup>4</sup>. Between 28 and 35 animals were added to each dish. Experiments were run for 1 h. In each experiment,  $\chi^2$  was applied to the numbers of preferring the two choices in the three dishes, in a  $2 \times 3$  contingency test. The  $\chi^2$  values for all three experiments were not significant, showing that within each experiment the results were homogeneous. The result from the three dishes in each experiment were therefore summed to give the data in the table.  $\chi^2$  was then applied to the numbers burrowed in the two choices in each experiment, and was significant: experiment 1,  $\chi^2 = 8.58$ , 1 d.f.  $0.005 > P > 0.001$ ; experiment 2,  $\chi^2 = 27.60$ , 1 d.f.  $P < 0.001$ ; experiment 3,  $\chi^2 = 8.02$ , 1 d.f.  $0.005 > P > 0.001$ . The number of animals not burrowed at the end of the experiment was small in experiment 1 and 2 (10 and 4% respectively), but larger in experiment 3 (35%) which is accounted for by the relative unsuitability of both autoclaved and irradiated sediments as assessed by experiments 1 and 2.

levels, which are marked with broad arrows in Fig. 1, were chosen for further experiments.

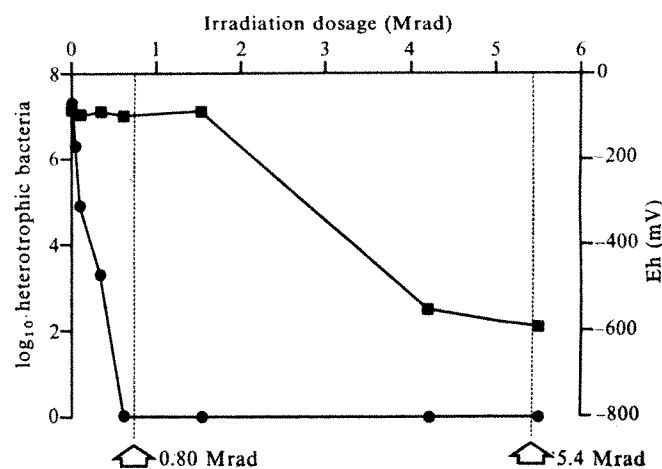
The behavioural response of *C. volutator* to 0.8-Mrad, 5.4-Mrad and control sediments was assessed (Table 2). *Corophium* could not distinguish between the control and the 0.8-Mrad sediments, but clearly preferred the 0.8-Mrad to the 5.4-Mrad sediment, and the control to the 5.4-Mrad sediment. The lack of difference between the control and 0.8-Mrad sediments shows that the absence of viable bacteria does not reduce the attractiveness of the sediments: in other words, *C. volutator* cannot distinguish between living and dead microorganisms. The marked avoidance by animals of the 5.4-Mrad sediments when compared with the 0.8-Mrad sediment or the control proves that animals avoid very low *Eh* values or some other associated change that occurs at high irradiation levels.

We believe that our results are relevant to the role played by microorganisms in habitat selection by benthic invertebrates<sup>5-9</sup>.

**Table 2** Preferences of *C. volutator* for control, 0.8-Mrad and 5.4-Mrad sediments

Expt	control sediment	% animals in 0.8-Mrad sediment	Total no. animals burrowed
(1)	53	47	93
Expt	control sediment	% animals in 5.4-Mrad sediment	Total no. animals burrowed
(2)	92	8	99
Expt	0.8-Mrad sediment	% animals in 5.4-Mrad sediment	Total no. animals burrowed
(3)	81	19	97

Each experiment was run in triplicate using three dishes<sup>4</sup>. Between 30 and 40 animals were added to each dish. Experiments were run for 1 h.  $\chi^2$  showed that the triplicate results were homogeneous and so the results for each experiment were summed to give the data in Table 2 (see legend to Table 1 for statistical details).  $\chi^2$  applied to the number of animals burrowing in the two choices in each experiment was not significant in experiment 1 ( $\chi^2 = 0.27$ , 1 d.f.  $0.7 > P > 0.5$ ) but highly significant in experiments 2 and 3 (experiment 2,  $\chi^2 = 69.59$ , 1 d.f.  $P < 0.001$ ; experiment 3,  $\chi^2 = 38.36$ , 1 d.f.  $P < 0.001$ ). In all three experiments very few animals had not burrowed by the end of the experiment (0-3%).

**Fig. 1** Sediment heterotrophic bacteria (●) and redox potential (■) in relation to irradiation dosage. The two broad arrows relate to the irradiation levels used in Table 2 (see text).

Most authors have stressed the importance of microorganisms as stimulus to sediment recognition, but their deductions have been based on the use of drastic physical and chemical treatments which alter the overall physico-chemical characteristics of sediments<sup>5</sup>. The lower of the two levels of irradiation used by us (0.8 Mrad, Fig. 1) does not seem to alter these characteristics but kills sediment microorganisms. Our findings show that in these circumstances *C. volutator* does not distinguish between living and dead cells (Table 2). We therefore conclude that the viability of sediment microorganisms may be of little significance in habitat selection and that their role lies in their effect on the chemical and physical properties of the sediment.

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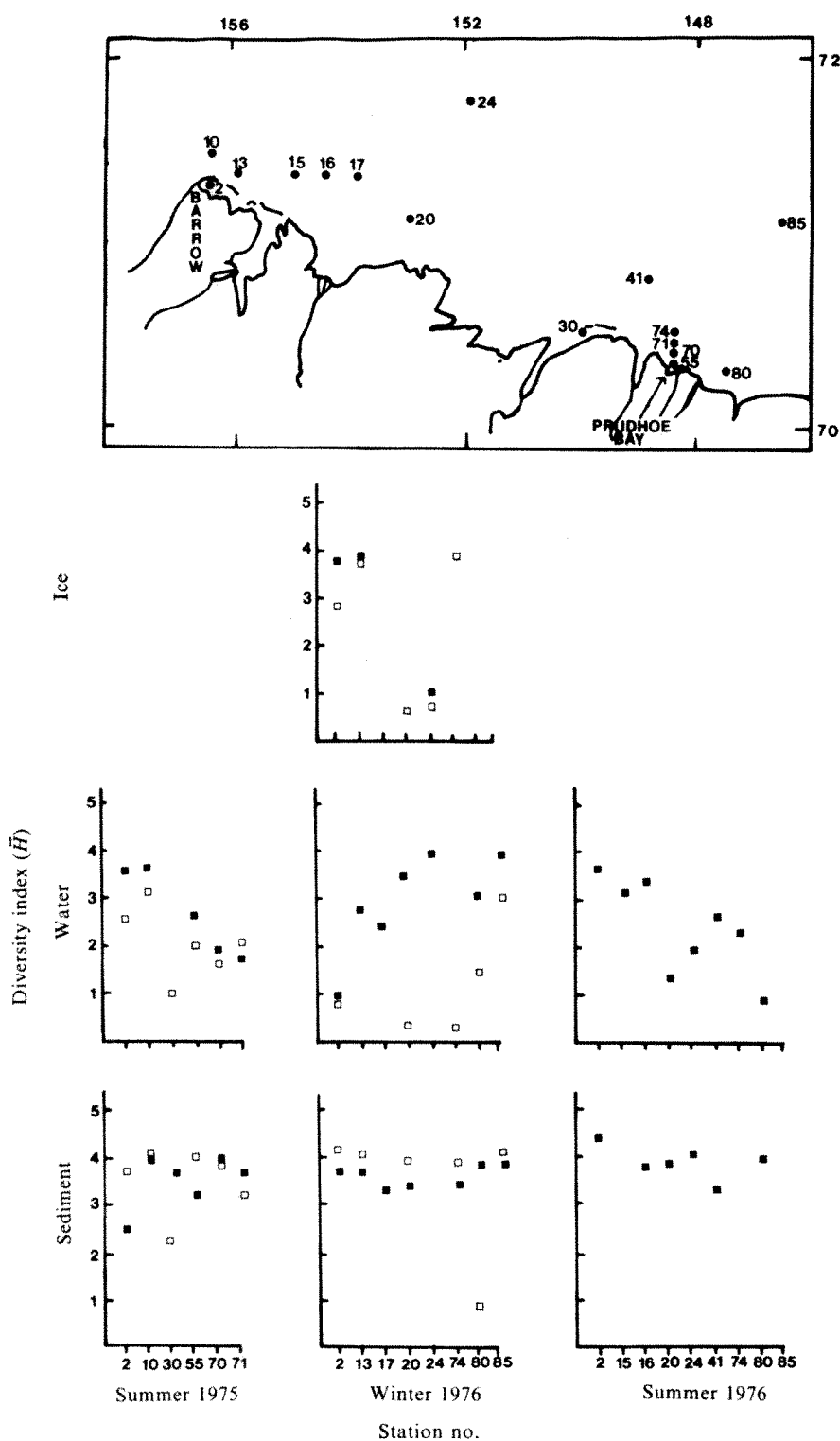
Received 2 June; accepted 18 October 1977.

1. Fenchel T. M. & Riedl, R. J. *Mar. Biol.* **7**, 255-268 (1970).
2. Meadows, P. S. & Anderson, J. G. *J. mar. biol. Ass. U.K.* **48**, 161-175 (1968).
3. Westheide, W. *Mar. Biol.* **1**, 336-347 (1968).
4. Meadows, P. S. *J. exp. Biol.* **41**, 499-511 (1964).
5. Meadows, P. S. & Campbell, J. I. *Adv. mar. Biol.* **10**, 271-382 (1972).
6. Gray, J. S. *Oceanogr. mar. Biol. A. Rev.* **12**, 223-261 (1974).
7. Scheltema, R. S. *Biol. Bull. mar. biol. Lab. Woods Hole* **120**, 92-109 (1961).
8. Wilson, D. P. *J. mar. biol. Ass. U.K.* **34**, 531-543 (1955).
9. Gray, J. S. & Johnson, R. M. *J. exp. mar. Biol. Ecol.* **4**, 119-133 (1970).

## Diversity of bacterial populations in the Beaufort Sea

BIOLOGICAL communities usually contain few species with many individuals and many species with few individuals<sup>1</sup>. Diversity of higher organisms generally has been found to decrease with increasing population size<sup>1</sup>. Communities in physically stressed ecosystems, such as in polar regions, characteristically have low species diversity<sup>2</sup>. Diversity indices have been used to express species diversity in plant and animal communities, especially as a measure of stress on community structure<sup>2</sup>. Amongst microorganisms, diversity indices have been applied to microscopic phytoplankton populations<sup>3,4</sup>, but not to bacterial populations. Bacterial populations, however, show large variations in species





**Fig. 1** Sampling sites in the Beaufort Sea and bacterial diversity indices during summer and winter in ice, water and sediment at these sites. Closed symbols, populations isolated at 4 °C, open symbols, populations isolated at 20 °C.

diversity. For example, infections are dominated by one or a few species, whereas many bacterial species are found in an ecosystem such as soil<sup>5</sup>. We report here the application of taxonomic diversity indices to bacterial populations and a study of the relationship of the diversity indices of such populations to population size and to population-independent factors such as geographic location.

It is difficult to define bacterial species and identify them in natural ecosystems. Unlike plants and animals, including even microscopic protozoa and algae, it is not possible to identify bacterial species by visual observation alone. We used numerical taxonomic procedures to define phenotypic clusters of bacteria, which we considered functionally equivalent to species. These clusters were used to calculate the species diversity of bacterial populations in nearshore areas of the Beaufort Sea.

Water and sediment samples were collected during late summer (August–September) 1975 and 1976 and late winter (March–April) 1976 at various sites in the western Beaufort Sea (Fig. 1). During winter, ice samples also were collected. Average surface water temperatures were about +1 °C in summer and –1.8 °C in winter. Samples were maintained at 4 °C during processing. Viable heterotrophic bacterial populations were enumerated by plating serial dilutions on marine agar 2216 (Difco). Duplicate series of plates were incubated at 4 °C for enumeration and isolation of psychrophilic–psychrotrophic populations, and at 20 °C for mesophilic populations. Details of these enumeration studies will be reported separately.

All of the bacterial colonies on the countable plates were isolated in pure culture. Bacterial isolates, 20–25 per sample, were selected at random for characterisation. Approximately 300

features were determined for each isolate, including morphological, physiological, biochemical and nutritional characteristics. The Jaccard coefficient ( $S_j$ ) and single linkage cluster analyses were used to sort the bacteria according to phenotypic similarity<sup>6</sup>. Strains related at greater than 70% similarity were considered to constitute a taxonomic grouping. Known strains of bacteria, included as controls, were separated into species at this level using the same testing procedures. The number of taxonomic groups and the number of individuals in each group, determined by the cluster analyses, were used to calculate the Shannon diversity index,  $\bar{H}$  (refs 7–10). The formula  $\bar{H} = c/N(N \log_{10} N - \sum n_i \log_{10} n_i)$  was used, where  $c = 3.3219$ ,  $N$  = total numbers of individuals and  $n_i$  = total number of individuals in the  $i$ th taxonomic grouping.

The diversity of the psychrophilic–psychrotrophic and mesophilic populations generally showed similar patterns of geographic variation (Fig. 1). Almost all of the bacterial strains were psychrotrophic or psychrophilic, as is found in most cold marine water and sediment samples<sup>11</sup>. Moving eastwards from Barrow towards Prudhoe Bay and northwards from Prudhoe Bay, bacterial diversity in water decreased during both summers, but increased during winter. No regular pattern of diversity between sampling sites was found in sediment or ice samples. Population levels in water were higher during summer 1975 and lower during winter 1976 near Prudhoe Bay than near Barrow. During summer 1976, populations were uniformly very high in all water samples.

Bacterial diversity in surface waters was inversely related to population size only at population levels below  $10^4 \text{ ml}^{-1}$  (Fig. 2). During summer 1976 when bacterial populations in water were above  $10^4 \text{ ml}^{-1}$ , no correlation between population size and

diversity was found. During summer, 1975, populations in water above  $10^4 \text{ ml}^{-1}$  generally had diversities of less than 2; populations less than  $10^4 \text{ ml}^{-1}$  had diversities of between 2 and 4 that were inversely related to population size.

During winter when bacterial populations in water were generally below  $10^4 \text{ ml}^{-1}$ , bacterial diversity clearly increased with decreasing population size. At the lower population levels generally found in water in winter, bacterial diversity was high. But, when population levels were similar in water samples collected in summer and winter, diversity was always lower in the winter samples. We consider the observed seasonal temperature variation of about  $2^\circ\text{C}$  insufficient to explain the observed differences in population size or diversity. Nutritional studies showed that these populations had extensive growth factor requirements. Presumably, these nutritional requirements are supplied by primary productivity of phytoplankton. Lack of light reaching the water during winter, thus may be a severe physical stress on the bacterial community, perhaps indirectly through phytoplankton productivity.

Compared with summer samples, a lower percentage of the strains isolated at  $4^\circ\text{C}$  from winter water samples, was capable of growth at  $20^\circ\text{C}$ . Selective pressure on mesophiles may account for differences in diversity between populations isolated at  $4^\circ\text{C}$  and  $20^\circ\text{C}$  during winter.

In sediment, bacterial populations were generally greater than  $10^4 \text{ g}^{-1}$ , regardless of sampling time.  $\bar{H}$  for bacterial populations in sediment was approximately 4, regardless of population size or sampling time. No correlation between population size and diversity was found for bacterial populations in sediment.  $\bar{H}$  for

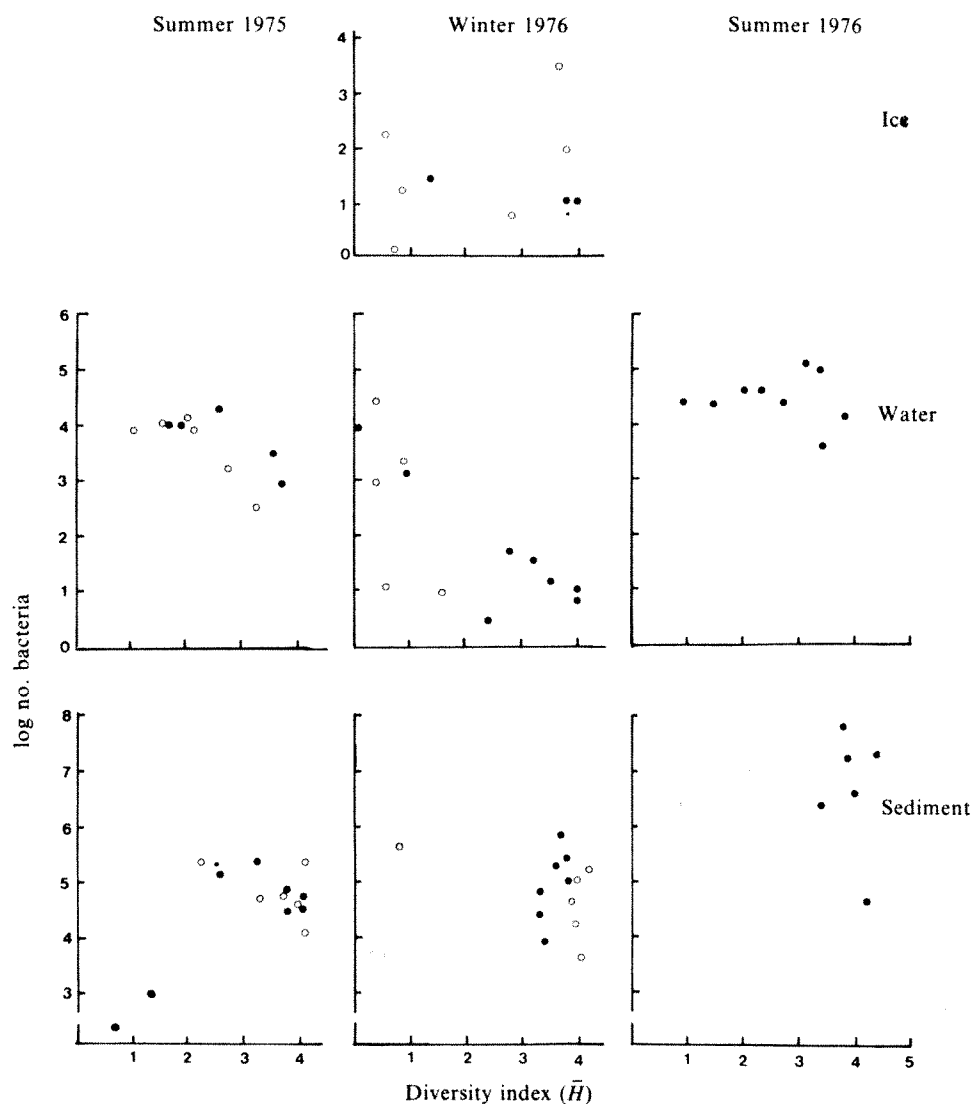


Fig. 2 Relation of population size to bacterial diversity in ice, water and sediment. Closed symbols, populations isolated at  $4^\circ\text{C}$ , open symbols, populations isolated at  $20^\circ\text{C}$ .

populations in sediment was generally higher than in water. Sediment populations are probably not subjected to the seasonal physical stress from temperature, salinity and light intensity changes as are water populations.

Although population levels were less than  $10^4 \text{ ml}^{-1}$  in sea ice samples, no correlation between population and diversity index was found for populations from ice. This probably reflects the fact that bacteria are frozen into the ice matrix and survive but do not grow. Therefore, selection pressures are not operative in ice.

In summary, numerical taxonomic testing of randomly selected bacterial strains can be readily used to calculate bacterial diversity. Bacterial diversity in Beaufort Sea water showed reciprocal geographic trends between summer and winter. While bacterial populations in water were generally lower during winter, as predicted, probably due to the physical stress, diversity at most sites remained as high as during summer. Bacterial populations up to a threshold population size showed an inverse relation between diversity and population size, as occurs in populations of higher organisms. Beyond the threshold size, the diversity index did not correlate with population size. Even above the threshold population size, though, the diversity index showed geographic differences in community structure for bacterial populations in water.

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*Note added in proof:* Since this manuscript was prepared Gainble, T. N., Bettlach, M. and Tiedje, J. M. applied diversity indices to denitrifying bacteria. *Appl. environ. Microbiol.* **33**, 926–939 (1977).

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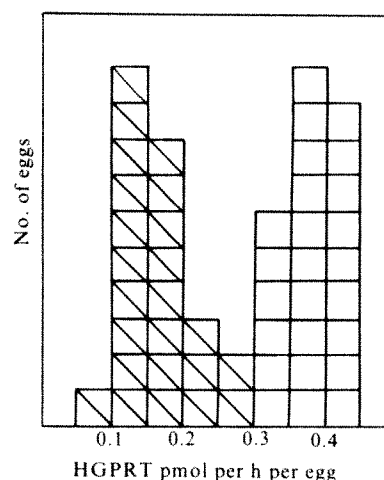
1. Fisher, R. A., Corbet, A. S. & Williams, C. B. *J. Anim. Ecol.* **12**, 42–58 (1943).
2. Odum, E. P. *Fundamentals of Ecology* **140**, (Saunders, Philadelphia, 1971).
3. Hurlbert, E. M. *J. mar. Res.* **21**, 81–93 (1963).
4. Patrick, R., Hohn, M. H. & Wallace, J. H. *Notul. natu. Acad. Philad.* **259**, 1–12 (1954).
5. Alexander, M. *Microbial Ecology* **10**, (Wiley, New York, 1971).
6. Sokal, R. & Sneath, P. H. A. *Principles of Numerical Taxonomy* (Freeman, San Francisco, 1963).
7. Shannon, C. E. & Weaver, W. *The Mathematical Theory of Communications* (University of Illinois Press, Urbana, 1949).
8. Margalef, R. *Perspectives in Ecological Theory* (University of Chicago Press, Chicago, 1968).
9. Pielou, E. C. *J. theoret. Biol.* **13**, 131–144 (1966).
10. Lloyd, M., Zar, J. H. & Karr, J. R. *Am. Mid. Nat.* **79**, 257–272 (1968).
11. Morita, R. *Bact. Rev.* **39**, 144–167 (1975).

## Dosage compensation for an X-linked gene in pre-implantation mouse embryos

THERE is strong evidence that one of the two X chromosomes is inactive in somatic cells of adult female mammals, so that the effective gene dosage per cell for X-linked loci is the same in XX females as in XY males or XO females. This system of dosage compensation is thought to be established soon after implantation<sup>1</sup>—certainly both X chromosomes seem to be potentially active in the blastocyst<sup>2</sup>. To test for dosage compensation before implantation one can study the activity of an X-chromosome-coded enzyme that is synthesised during pre-implantation development, as a result of *de novo* transcription, in male and female embryos. Two such enzymes are considered to be hypoxanthine phosphoribosyl transferase (HGPRT EC 2.4.2.8) and  $\alpha$ -galactosidase<sup>3,4</sup>. Epstein<sup>3</sup> found that groups of unfertilised

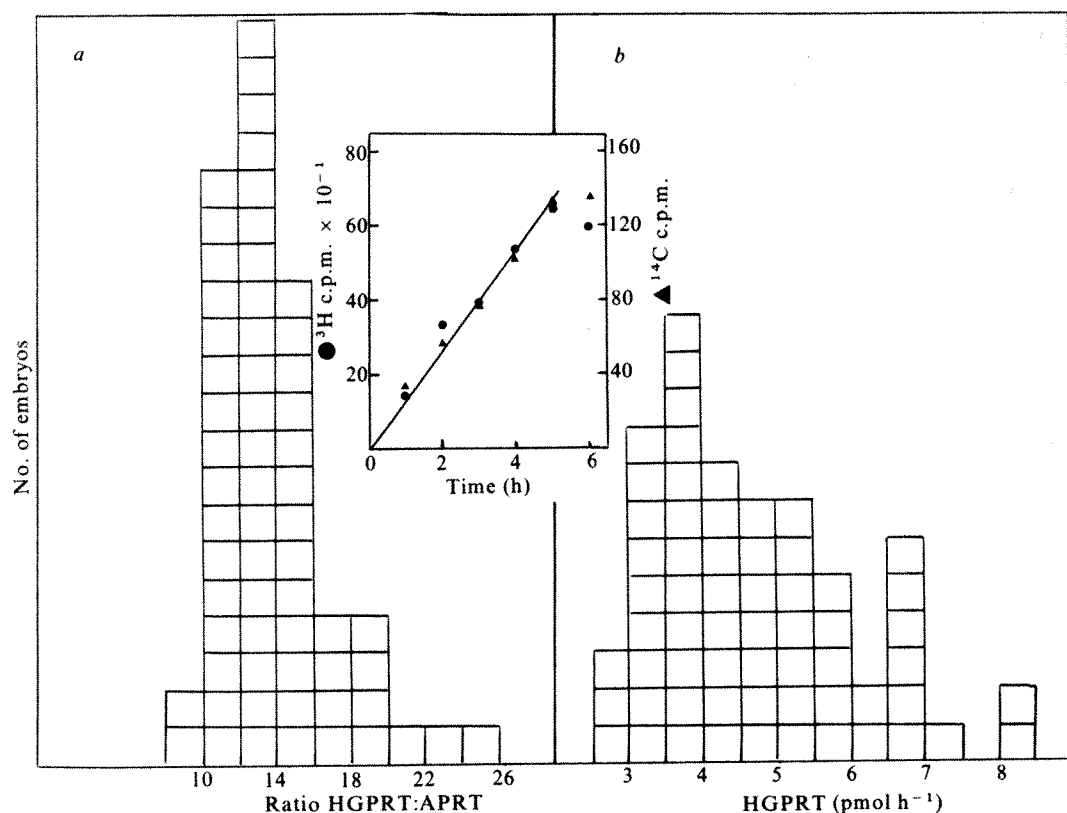
eggs from XX mothers had twice the HGPRT activity found in those from XO mothers, indicating that both X chromosomes are functional during oogenesis<sup>3,5</sup>. By the morula and blastocyst stage, HGPRT activities had increased strikingly and the twofold difference had disappeared, implying that the enzyme present was no longer maternal in origin. Because pooled male and female embryos were assayed it was impossible to determine whether dosage compensation was occurring. Adler *et al.*<sup>6</sup> measured  $\alpha$ -galactosidase activity in single pre-implantation embryos, expecting that the presence of both female and male embryos would give rise to either a bimodal or a unimodal distribution of activities in the absence, or presence, respectively, of dosage compensation. The assay was insufficiently precise to give unequivocal results. We have now developed a single-embryo assay for HGPRT of sufficient precision to establish that the distribution of activities in single embryos, half of which we expect to be XX and half XY, is unimodal at both the eight-cell and the blastocyst stage. We conclude that dosage compensation occurs during pre-implantation development.

For single-embryo assays, where variation in the recovery of cytoplasmic contents would be expected to contribute substantially to the variance of the activity measurements, increased accuracy is achieved by the simultaneous measurement of activities of HGPRT and of an autosomal enzyme (adenine phospho-ribosyl transferase, EC 2.4.2.7) in the same reaction mixture. The results are expressed as a ratio of HGPRT:APRT activities in the individual



**Fig. 1** Assays of HGPRT were carried out on batches of five to 10 unfertilised eggs from XX and XO (hatched squares) females (genotypes Ta/+ and +/O). Superovulation was induced by intraperitoneal injection of 7.5 IU of pregnant mare serum and 48 h later, 7.5 IU of human chorionic gonadotrophin. Eggs were isolated from the oviducts on the next morning, cumulus cells were removed with hyaluronidase and the eggs were washed carefully several times. The media used for collection of eggs and embryos (Figs 2 and 3) and the methods of isolation are described in ref. 8. The batches of eggs were transferred in less than 5  $\mu$ l of PB1 medium into individual microcaps and extracts were prepared by freeze-thawing in liquid nitrogen three times, followed by centrifugation at 2,000 r.p.m. for 10 min. The supernatants were added to 50  $\mu$ l volumes of reaction mixture. Reaction mixtures contained (final concentration) 35 mM sodium phosphate buffer (pH 7.4), 5 mM magnesium chloride, 2 mM phosphoribosyl pyrophosphate, 10  $\mu$ M <sup>3</sup>H-guanine (specific activity 0.084 Ci mmol<sup>-1</sup>) and 10  $\mu$ M <sup>14</sup>C-adenine (specific activity 58 mCi mmol<sup>-1</sup>). The reactions were carried out at 37 °C for 3 h and stopped by addition of 1 ml of 0.1M ice-cold lanthanum chloride containing 1 mM adenine and 1 mM guanine. Extracts from 25 batches of XX eggs and 24 batches of XO eggs were assayed individually; the mean activities of HGPRT (pmol per egg per h  $\pm$  s.e.) were  $0.383 \pm 0.001$  and  $0.164 \pm 0.002$ , respectively. The mean activities of APRT (pmol per egg per h  $\pm$  s.e.) were  $0.17 \pm 0.01$  and  $0.21 \pm 0.01$  for XX and XO eggs, respectively (data not shown).





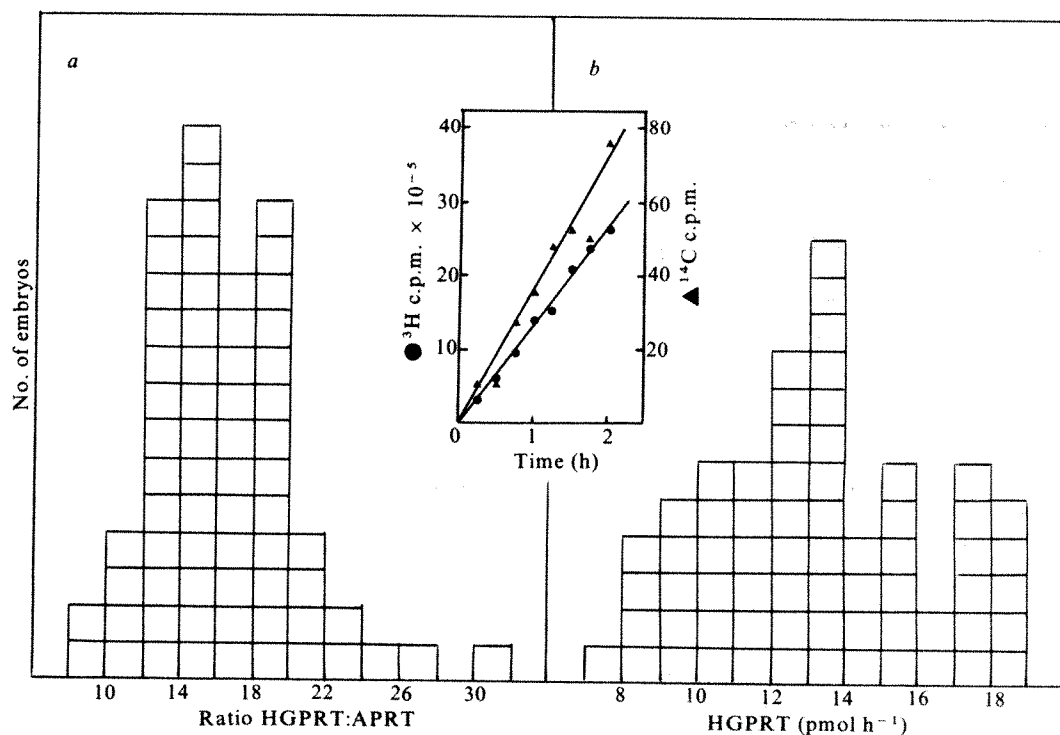
**Fig. 2a**, Ratio of activities of HGPRT to APRT in 62 single eight-cell embryos isolated from oviducts from MF1 females (Olac 1976 Ltd) on the third day of pregnancy. Each square represents the ratio obtained for a separate eight-cell embryo. Individual embryos were isolated into microcaps. The preparation of embryo extracts and the assay procedure are described in the legend to Fig. 1. Assays were carried out at 37 °C for 4 h. Data from two experiments are pooled. The mean HGPRT and APRT activities (pmol per h per embryo  $\pm$  s.e.) were  $4.697 \pm 0.172$  and  $0.346 \pm 0.013$ , respectively. The inset figure shows both enzyme reactions to be linear in eight-cell embryos for at least 5 h. **b**, Activities of HGPRT in single eight-cell embryos.

embryo. The simultaneous assay of the two enzymes is based on the conversion by cell-free extracts of <sup>3</sup>H-guanine and <sup>14</sup>C-adenine to their respective monophosphates<sup>6</sup>. These are precipitated with lanthanum chloride and collected on filters, and the ratio of the two isotopes is determined.

To establish the validity of our assay system we first measured HGPRT activity in groups of unfertilised eggs from XX and XO females and confirmed Epstein's findings<sup>3</sup>

of an approximate twofold difference (Fig. 1), whereas the activity of APRT was nearly the same (see legend to Fig. 1). As further controls, we assayed extracts of cell lines lacking HGPRT (for example, an HGPRT-deficient derivative of PSA4<sup>7</sup>); these gave no incorporation of <sup>3</sup>H-guanine into precipitable product, though high APRT levels were found. Also, the omission of the substrate, phosphoribosyl pyrophosphate (PRPP) from the single-embryo

**Fig. 3a**, Ratios of activities of HGPRT to APRT in 67 single blastocysts isolated from the uteri of MF1 females on the fourth day of pregnancy. Each square represents the ratio obtained for a separate blastocyst. Individual blastocysts were isolated into microcaps. The preparation of extracts and the assay procedure are described in the legend to Fig. 1. Assays were carried out at 37 °C for 2 h. The data from two separate experiments are pooled. The mean HGPRT and APRT activities (pmol per h per embryo  $\pm$  s.e.) were  $13.269 \pm 0.367$  and  $0.822 \pm 0.021$ , respectively. The inset shows that both enzyme reactions are linear in blastocyst extracts for at least 2 h (the specific activity of the <sup>3</sup>H-guanine in this timed experiment was 8.4 Ci mmol<sup>-1</sup> instead of 0.084 Ci mmol<sup>-1</sup>). **b**, Activities of HGPRT in single blastocysts.



assays inhibited both enzymatic reactions. Such factors as breakdown of substrates, nucleotide pool sizes and enzymes which may destroy the nucleotide products, may well vary between stages of development, but would not be expected to differ between female and male embryos at a comparable stage.

The results for a series of single eight-cell embryos and single blastocysts are given in Figs 2 and 3. The variances of the distributions are relatively small, and are not consistent with a bimodal distribution, corresponding to two populations of equal size with modes differing by a factor of two. Even the distributions for HGPRT activities alone (Figs 2b and 3b) are not consistent with such a model. The mean levels of activity are in good agreement with those obtained by Epstein<sup>3</sup> using another assay on groups of embryos. At the eight-cell stage, the earliest stage at which single-embryo assays are practicable, the activity levels are low, and may merely reflect the levels of maternal mRNA. By the blastocyst stage, however, activity levels are 30-fold higher than at fertilisation and embryonic gene action at the HGPRT locus is established by Epstein's work<sup>3</sup>. We therefore conclude that some form of dosage compensation operates in most, if not all, cells of the pre-implantation blastocyst. The trophectoderm constitutes most of the cells of the blastocyst but further analysis is required to establish whether dosage compensation operates within the cells of the inner cell mass as well as in those of the trophectoderm.

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1. Lyon, M. F. *Nature* **190**, 372-373 (1961); *Biol. Rev.* **47**, 1-35 (1972).
2. Gardner, R. L. & Lyon, M. *Nature* **231**, 385-386 (1971).
3. Epstein, C. J. *Science* **163**, 1078-1079 (1969); *J. biol. Chem.* **245**, 3289-3294 (1970); *Science* **175**, 1467-1468 (1972).
4. Adler, D. A., West, J. D. & Chapman, V. M. *Nature* **267**, 838-839 (1977).
5. Kozak, L. P., McLean, G. K. & Eicher, E. M. *Biochem. Genet.* **11**, 41-47 (1974).
6. McBurney, M. W. & Adamson, E. D. *Cell* **9**, 57-90 (1976).
7. Martin, G. R. & Evans, M. J. in *Teratocarcinoma and Differentiation* (ed. Sherman, M. I. & Solter, D.) 169-187 (Academic, New York, 1975).
8. Monk, M. & Ansell, J. *J. Embryol. exp. Morph.* **36**, 653-662 (1976).

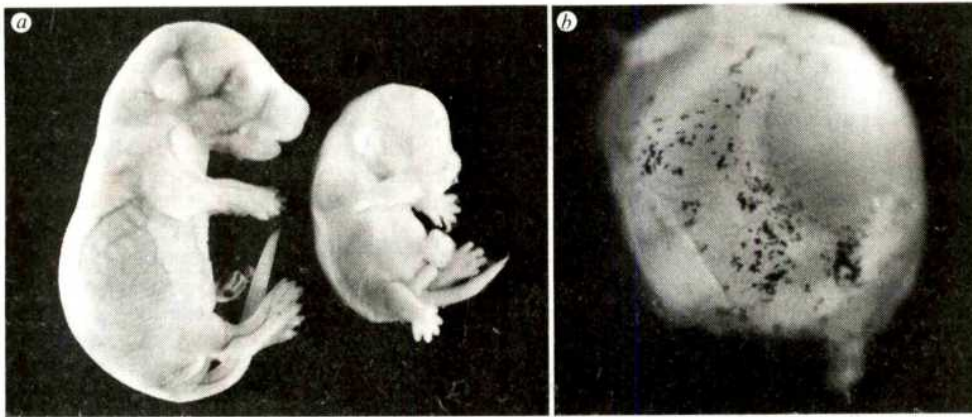
## Development to term of chimaeras between diploid parthenogenetic and fertilised embryos

PARTHENOGENETIC mouse embryos have the potential for extensive cellular proliferation as well as differentiation into various cell types<sup>1-3</sup>. But this potential has been realised only when parthenogenetic embryos have been transferred to extrauterine sites<sup>1</sup>, and in spontaneously occurring ovarian teratomas and teratocarcinomas of parthenogenetic origin<sup>2,3</sup>. The development of mammalian parthenogenetic embryos *in utero* is restricted, with no conclusive evidence that they can develop to term<sup>4,5</sup>. Several hypotheses have been proposed to account for their poor development. For example, deleterious recessive genes may affect the viability of their cells, possibly because of their extensive homozygosity<sup>2,3</sup>, or disorganised growth and limited life span may result from anomalies of cellular interactions<sup>6</sup>. But the extensive cellular proliferation and differentiation of parthenogenetically derived cells which occurs in extrauterine sites is not entirely consistent with

these explanations, and indicates that parthenogenones probably have a relatively stable genetic constitution. Indeed, these studies stress the likely importance of cellular environment for cytodifferentiation, provided in this instance by the extrauterine host tissue. There is a precedent for supposing that if the environment is critical for cytodifferentiation, parthenogenetic cells should be able to form chimaeras with cells derived from fertilised embryos<sup>6</sup>. Teratocarcinoma cells<sup>7,8</sup> and cells carrying known lethal alleles<sup>9,10</sup> can develop into viable chimaeras when aggregated with cells from normal embryos. Previous attempts to achieve development to term of aggregation chimaeras between parthenogenetic and fertilised embryos were apparently unsuccessful<sup>11</sup>. We have introduced inner cell masses (ICMs) from diploid parthenogenetic embryos into intact fertilised mouse blastocysts, and we report here the development of a chimaera to term.

Mouse oocytes isolated 18 h after injection of human chorionic gonadotrophin for superovulation from (C57BL × CBA) F<sub>1</sub> females were activated *in vitro* in medium lacking Ca<sup>2+</sup> and Mg<sup>2+</sup> salts<sup>5,12</sup>, and the diploid parthenogenetic embryos allowed to develop to the blastocyst stage in culture<sup>3</sup>. Of 397 activated eggs 37 (14%) had one pronucleus and the second polar body; 160 (60.6%) had two pronuclei without the second polar body; eight (3%) had one pronucleus without the second polar body, and 59 (22.3%) went into immediate cleavage. The overall activation frequency was 66.5%. The embryos were homozygous for the isozyme of glucose phosphate isomerase (GPI), *Gpi-1<sup>b</sup>/Gpi-1<sup>b</sup>* and carried coat colour and eye colour genes of the C57BL and CBA strains. Of the 160 activated eggs with two pronuclei without the second polar body, 81 (50.6%) reached the blastocyst stage. We obtained 43 ICMs from the blastocysts and were able to transfer 20 to recipient blastocysts. ICMs were isolated by a technique involving exposure of blastocysts to the calcium ionophore A23187. The parthenogenetic blastocysts were exposed to A23187 at the final concentration of 2 × 10<sup>-3</sup> M for 20-30 min. This caused selective lysis of trophectoderm cells, leaving the ICM cells intact (M.A.H.S., D. Torchiana and S.C.B., in preparation). Individual ICMs were introduced microsurgically<sup>13</sup> into blastocysts on the 4th day of pregnancy obtained by matings of *Gpi-1<sup>a</sup>/Gpi-1<sup>a</sup>* albino CFLP random-bred mice (Anglia Laboratory Animals Ltd). After 3-4 h culture at 37 °C, blastocysts were transferred to the uterus of d3 pseudopregnant CFLP mice previously mated to proven sterile vasectomised males (the day when vaginal plug was found was d1 of pseudopregnancy). Four blastocysts were transferred to each of the five recipients.

One female examined on d9 of pregnancy contained four implantation sites. Four apparently normal early somite embryos were recovered. The embryos and their respective ectoplacental cones were separately typed for GPI<sup>14</sup>. All four embryos were chimaeric, whereas the ectoplacental cones only contained the *Gpi-1<sup>a</sup>* isozyme band. Two embryos probably contained 40-50% contribution from parthenogenetic cells, whereas the other two contained between 5 and 30% contribution from parthenogenetic cells. This assessment was made subjectively from the intensity of staining *Gpi-1<sup>b</sup>* and *Gpi-1<sup>a</sup>* isozyme bands (Table 2). The other four females were autopsied on d19 of pregnancy. Three of them were not pregnant, having no evidence of implantation sites. One female contained two embryos (Fig. 1a). The smaller embryo was dead, although morphologically an apparently normal male. This embryo showed pigmentation of the sclera and iris and had probably survived up to d15-17 of pregnancy. The larger embryo was alive at autopsy, and initiated spontaneous respiratory movements. Attempts were made to foster the foetus, but it was unfortunately not accepted by the foster mother. Anatomical examination of this embryo showed it to be an apparently



**Fig. 1** *a*, Chimaeric embryos isolated from d19 pregnant female. The larger embryo was alive at autopsy and was an apparently normal female. The smaller embryo was dead and had probably developed until d15–17 of pregnancy and was an apparently normal male. *b*, An eye dissected from the larger embryo showing pigmentation of the choroid. The iris and retina were also pigmented.

normal female. The eyes were dissected out and showed pigmentation of the choroid (Fig. 1*b*) and iris, as well as the retina. All major organs from the two embryos were typed for GPI and found to be chimaeric. Between 40 and 50% of the cells in the smaller, dead embryo were of parthenogenetic origin, while in the live embryo the contribution was considerably smaller, being in the region of 10–25% (Table 1).

function normally<sup>15,16</sup>. Similar cellular interactions between parthenogenetic and normal fertilised cells may also ensure cooperation of parthenogenetic cells in normal development.

These results suggest that it should be possible to obtain mature adult chimaeras. This would enable us to examine whether the parthenogenetically derived cells are fully differentiated in these chimaeras, and to examine whether they contribute to the normal functioning of all tissues and

**Table 1** The contribution made by parthenogenetic cells in chimaeras assessed from GPI typing

Embryo no.	Day of pregnancy at autopsy	Description of embryos	Embryo (excluding membranes)	Ecto-placental cone	Tissues examined for parthenogenetic cells (%)*									
					Blood	Liver	Lung	Brain	Heart	Gonad	Kidney	Amnion	Eye	Carcass
1	d9	10–15 somite stage	40	ND	—	—	—	—	—	—	—	—	—	—
2	"	"	5	ND	—	—	—	—	—	—	—	—	—	—
3	"	"	50	ND	—	—	—	—	—	—	—	—	—	—
4	"	"	30	ND	—	—	—	—	—	—	—	—	—	—
5	d19	d15–17, male dead	—	—	NE	NE	NE	NE	NE	40	NE	NE	50	50
6	d19	d19, female live	—	—	25	10	15	10	10	15	10	15	15	15

ND, not detected; NE, not examined, tissue unsuitable.

\*Values represent subjective assessment from GPI analysis based on the time of appearance of the *Gpi-1*<sup>a</sup> and *Gpi-1*<sup>b</sup> bands and the relative intensities of the two bands<sup>14</sup>.

These results demonstrate that cells from diploid parthenogenones, in addition to their potential for proliferating and differentiating in ectopic sites, can participate with cells from fertilised embryos in the development to term of an apparently normal chimaeric embryo. We tentatively suggest that a large number of such chimaeras may be able to develop to term when the parthenogenetic contribution is probably not more than 20% of the total cell population in the individual tissues and organs. The survival and integration of parthenogenetic cells in such chimaeras is probably largely influenced by the environmental conditions determined by the cells from the fertilised embryo, in contrast to the extensive but haphazard cell growth and differentiation which occurs when parthenogenetic and fertilised embryos are transferred to ectopic sites<sup>1</sup>. The exact nature of this environmental influence remains unclear, but there are several probable ways in which such influences may be mediated. For example, exposed sites at the cell surface and/or secreted molecules may compensate for any deficiencies in parthenogenetic cells, permitting their normal development and differentiation. This type of influence may be involved in the 'rescue' of androgen-resistant germ cells<sup>9</sup>. • There is evidence for metabolic cooperation between genetically diverse cell types through permeable junctions which enables metabolically deficient cells to

organs including the germ cells. This study also demonstrates that the presence of a genetic or extragenetic contribution from the male may not be a prerequisite for the normal development and differentiation of mammalian cells. Experiments are in progress to establish the differentiation potential of both haploid and diploid parthenogenetic cells.

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*Note added in proof:* One apparently normal chimaeric male which is now 4-weeks-old, has been obtained after further transfers of six operated blastocysts. Similar chimaeras have recently been reported by Stevens *et al.*<sup>17</sup>.

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1. Iles, S. A., McBurney, M. W., Bramwell, S. R., Deussen, Z. A. & Graham, C. F. *J. Embryol. exp. Morph.* **34**, 387–405 (1975).
2. Stevens, L. C. in *The Developmental Biology of Reproduction, symp. Society Dev. Biol.* 93–106 (Academic, New York, 1975).
3. Stevens, L. C. & Varnum, D. S. *Dev. Biol.* **37**, 369–380 (1974).
4. Beatty, R. A., *Parthenogenesis and Polyploidy in Mammalian Development* (Cambridge University Press, Cambridge, 1957).
5. Kaufman, M. H., Barton, S. C. & Surani, M. A. H. *Nature* **265**, 53–55 (1977).
6. McLaren, A. *Mammalian Chimaeras* (Cambridge University Press, Cambridge, 1976).
7. Papaioannou, V. E., McBurney, M. W., Gardner, R. L. & Evans, M. J. *Nature* **258**, 71–73 (1975).
8. Mintz, B. & Illmensee, K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3585–3589 (1975).
9. Lyon, M. F., Glenister, P. H., Lamoreux, M. H. *Nature* **258**, 620–622 (1975).
10. Mintz, B. *Ann. Rev. Genet.* **8**, 411–470 (1974).
11. Graham, C. F. *Nature* **226**, 165–167 (1970).
12. Surani, M. A. H. & Kaufman, M. H. *Dev. Biol.* **59**, 86–90 (1977).
13. Gardner, R. L. *Adv. Biosci.* **6**, 279–296 (1971).
14. Chapman, V. M., Whitten, W. K. & Ruddle, F. H. *Dev. Biol.* **26**, 153–158 (1971).
15. Subak-Sharpe, H., Burk, R. R. & Pitts, J. D. *J. Cell Sci.* **4**, 353–367 (1969).
16. Pitts, J. D. & Burk, R. R. *Nature* **264**, 762–764 (1976).
17. Stevens, L. C., Varnum, D. S. & Eicher, E. M. *Nature* **269**, 515–517 (1977).

## Amiloride-sensitive rheogenic $\text{Na}^+$ transport in rabbit blastocyst

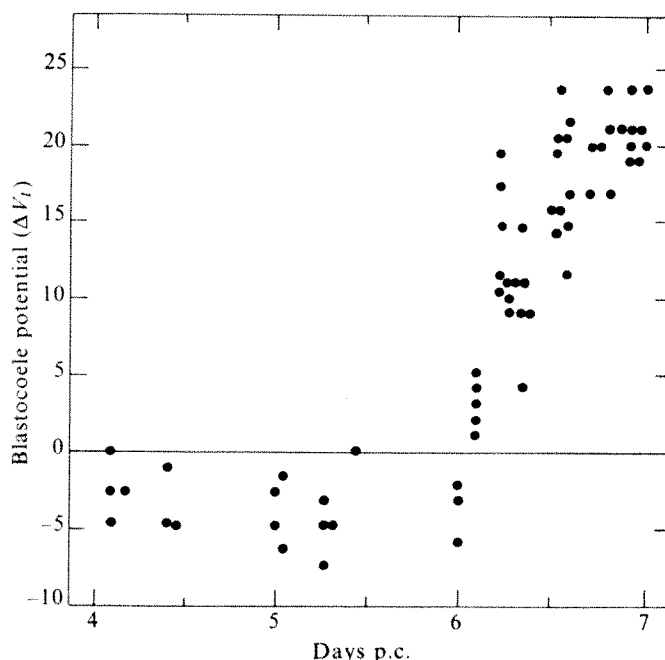
FLUID and solutes accumulate in the blastocyst just before implantation by transport mechanisms associated with the trophectoderm<sup>1–3</sup>. This structure is a simple squamous epithelium that surrounds the embryo, and it is the first tissue to develop in the mammalian embryo<sup>4</sup>. We describe here electrophysiological experiments which demonstrate changes in transport functions of this epithelium in the rabbit a few hours before the expected time of implantation. At this time fluid is accumulating rapidly in the blastocoele<sup>5</sup>. The following results show that between 6 and 7-d post-coitum (p.c.) a transtrophectodermal, rheogenic, amiloride-sensitive  $\text{Na}^+$  transport system develops. The term 'rheogenic' characterises non-neutral or current-generating transport process, as opposed to the term 'electrogenic' which is more general and characterises any process (diffusion potentials, electrokinetic phenomena, and/or rheogenic transport) which results in a change in potential across an epithelium<sup>6</sup>.

The electrical potential difference between the blastocoele cavity and the external medium, the transtrophectodermal potential difference ( $\Delta V_t$ ) was measured in 4-, 5-, 6- and 7-d p.c. rabbit embryos. The electrophysiological techniques used to make the measurements were the same as those used in studies on the oocyte<sup>7</sup>, and the methods of obtaining and culturing the rabbit embryos were the same as used in former studies<sup>8</sup>. The measurements were made while the blastocysts were maintained at 37 °C on the heated stage of a microscope with continuous monitoring of the pH. The  $\Delta V_t$  did not change between pH 7.4 and 7.6 and the media were maintained between these values. The embryos were incubated in a modified F10 medium<sup>9,10</sup> that consisted of Ham's F10 medium (Microbiological Associates) buffered with 0.02 M HEPES (Calbiochem) containing 20% foetal calf serum and adjusted to pH 7.4 with 1 N NaOH. As shown in Fig. 1 there is a large change in the  $\Delta V_t$  between 4 and 7 d p.c. with most of the change occurring between 6.0 and 6.5 d p.c.

The membrane potential of the trophoblast cells which surround the blastocoele was also measured. Because these cells are extremely thin it proved difficult to obtain long term penetrations (> 1 min). But the data from 30 trophoblast cells from eight 5-d blastocysts and 51 trophoblast cells from 15 6–7-d blastocysts demonstrate a significant change in electrical potential from  $-34.0 \pm 4$  to  $-46.7 \pm 3.1$  mV. The basis for this change has not been examined.

The effect of the two extracellular coats on  $\Delta V_t$  was examined. The mucin coat, which is found on the 4-d p.c. blastocyst, caused no alteration of potential when the microelectrode was placed inside it, but outside the trophoblast layer. The zona pellucida was likewise found to have no effect. It was removed from the 5- and 6-d p.c. embryo by Pronase (0.25%) without causing change in the  $\Delta V_t$ .

The results obtained on the  $\Delta V_t$  are at variance with those reported by Cross and Brinster for the 5-, 6- and 7-d p.c. rabbit blastocyst measured *in vitro* in Kreb's Ringer bicarbonate with



**Fig. 1** Developmental changes in the transtrophectodermal potential difference ( $\Delta V_t$ ) measured in freshly collected rabbit blastocysts on days 4, 5, 6 and 7 p.c. The  $\Delta V_t$  were measured at 37 °C in modified F10 medium supplemented with 20% foetal calf serum. The pH of the medium was 7.4.

glucose (KRBG) ( $-7.6$  mV,  $-11.9$  mV,  $-2.5$  mV, respectively, blastocoele negative with respect to the medium) and for the 6-d p.c. blastocyst ( $-6.0$  mV) in Eagle's medium with 20% foetal calf serum<sup>11</sup>. Our measurements show that the  $\Delta V_t$  in 4 and 5-d p.c. blastocysts is negative ( $-3.14$  mV and  $-4.38$  mV), but by 6.5-d p.c. the  $\Delta V_t$  rises to  $+14.5$  mV and at 7 d reaches  $+21$  mV (Table 1). This change in polarity is found both in embryos cultured *in vitro* from day 5 and those allowed to remain in the uterus until day 6. Measurements were not attempted beyond day 7, the normal time for implantation, as the blastocysts become very fragile in culture beyond that stage.

In order to resolve the discrepancy with Cross and Brinster's results the effect of different media on the measured potential was examined. Medium F10 with 20% foetal calf serum for rabbit embryo culture was used routinely as it has been shown to be more effective than KRBG for long term culture<sup>9,10</sup>. Cross and Brinster measured the  $\Delta V_t$  throughout development in KRBG, but, as shown in Table 1, this does not account fully for the difference in measured values. We do find a significant reduction, however, in the  $\Delta V_t$  of 6.5-d p.c. blastocysts when they are moved from F10 to KRBG. This reduction may relate to a reduction of energy available for ion transport, in  $\text{Na}^+$  coupled amino acid or carbohydrate transport, or an alteration in membrane ion permeability. The data suggest that studies on blastocyst transport should be performed on identical age embryos to avoid developmental changes which will make results ambiguous.

The  $\Delta V_t$  was also measured in KRBG after various ion substitutions. On day 5 p.c. the  $\Delta V_t$  changes to a positive potential in Cl-depleted medium, whereas replacement of  $\text{Na}^+$  with choline results in an increase in the  $\Delta V_t$ .  $\text{NaCl}$ -free medium caused a reversal in polarity of the  $\Delta V_t$ . The response of the  $\Delta V_t$  is in the direction predicted by the electrochemical gradients for these ions<sup>8</sup>, and the results agree with those reported by Cross and Brinster<sup>11</sup>. On day 6.5 p.c.  $\text{Na}^+$ -free medium causes a larger decrease in the  $\Delta V_t$ . Cl-depleted medium caused a slight increase in  $\Delta V_t$  but this increase was not statistically significant.

Amiloride has been extensively used to analyse  $\text{Na}^+$  transport in epithelia<sup>12–14</sup> and it is presumed to act by blocking passive  $\text{Na}^+$  entry into epithelial cells. No measureable effect of amiloride in the 5-d blastocyst in concentrations up to 1 mM in KRBG has been found. But, the  $\Delta V_t$  of 6.5-d p.c. blastocysts is amiloride-sensitive

**Table 1** Transtrophectodermal potential difference of the rabbit blastocyst

Medium	Age (d p.c.)			
	4	5	6.5	7
F10	$-3.14 \pm 0.77$ <i>n</i> = 7	$-4.38 \pm 0.68$ <i>n</i> = 8	$+14.5 \pm 1.0$ <i>n</i> = 26	$+21.0 \pm 0.8$ <i>n</i> = 15
KRBG		$-3.8 \pm 0.8$ <i>n</i> = 10	$+6.0 \pm 2.1$ <i>n</i> = 8	
Na <sup>+</sup> -free		$-9.1 \pm 1.2$ <i>n</i> = 8	$-15.2 \pm 3.2$ <i>n</i> = 6	
KRBG		$+6.67 \pm 1.0$ <i>n</i> = 6	$+9.0 \pm 2.3$ <i>n</i> = 5	
Cl <sup>-</sup> -free		$+3.3 \pm 1.1$ <i>n</i> = 6		
KRBG				
Amiloride in				
KRBG		$-4.12 \pm 0.37$ <i>n</i> = 6	$-3.25 \pm 0.65$ <i>n</i> = 8	$-3.85 \pm 0.9$ <i>n</i> = 5
(10 $\mu$ M)				

The electrical potential of the blastocoele is given in mV positive or negative ( $\pm$  s.e.m.) with respect to the bathing medium. KRBG contains the following solutes (mM): 119 NaCl, 4.74 KCl, 1.71 CaCl<sub>2</sub>, 1.19 KH<sub>2</sub>PO<sub>4</sub>, 1.19 MgSO<sub>4</sub>, 7H<sub>2</sub>O, 25 NaHCO<sub>3</sub> and 5.55 glucose. In Na-free medium, NaCl was replaced with equimolar choline chloride and NaHCO<sub>3</sub> was replaced with choline bicarbonate. Na<sub>2</sub>SO<sub>4</sub> replaced NaCl, and K<sub>2</sub>SO<sub>4</sub> replaced KCl in Cl-depleted medium. In NaCl-free medium, choline methyl sulphate replaced NaCl and choline bicarbonate replaced NaHCO<sub>3</sub>. The osmolarities of the ionically substituted media were adjusted with sucrose, and the pHs were brought to 7.4.

and can be reduced in a dose dependent fashion to approximately  $-3$  mV by raising the concentration of amiloride in the medium to 10  $\mu$ M. The response of the 6–7-d p.c. embryo to amiloride is maximum at 10  $\mu$ M, is directly proportional to the magnitude of the positive  $\Delta V_i$  and is immediate. These findings provide strong evidence that the increase in  $\Delta V_i$  seen between 5 and 7-d p.c. is the result of the onset of rheogenic Na<sup>+</sup> transport. This mechanism is, in all likelihood, responsible for the increase in blastocoele Na and Cl which occurs during this period<sup>8</sup>, and Cross<sup>15</sup> obtained evidence suggesting that Na and Cl influxes into the 6-d p.c. rabbit blastocoele may be coupled to each other. This hypothesis is consistent with our results on the 4 and 5-d embryo, but not with the positive  $\Delta V_i$  that begins at 148 h p.c.

The current generating mechanism reflects the movement of a charged species across a barrier which has electrical resistance. The potential generated across the epithelium is directly proportional to the resistance of the epithelium and the rate at which ions are actively moved across the barrier. The change in potential which is observed between 6 and 7 d p.c. may, therefore, be caused by either an increase in the resistance of the trophoctoderm or an increase in Na<sup>+</sup> influx into the blastocoele. Cross<sup>15</sup> calculated a resistance of 2,742  $\Omega$  cm<sup>2</sup> for the 6-d blastocyst from short-circuit current measurements. Because of microelectrode tip resistance, the large surface area of the 6-d blastocyst and the greatly decreased total resistance of the trophoctoderm, we have not been able to measure the resistance of highly expanded blastocysts on day 6. Blastocysts on day 4 p.c. are small and have a large total resistance compared to the microelectrode's tip resistance. Measurements of the electrical resistance of eight blastocysts on day 4 p.c. indicate that the rabbit trophoctoderm has a resistance of  $3,117 \pm 400$   $\Omega$  cm<sup>2</sup>. These data, as well as 5–8 tight junctions on the apical surface of the trophoblast cells<sup>16,17</sup>, and the impermeability of the trophoctoderm to lanthanum<sup>16</sup>, support the idea that the trophoctoderm is a tight epithelium. Because of the uncertainties in the measurements of resistance on the 6 and 7-d blastocyst a definitive statement about the mechanism(s) causing rheogenic Na<sup>+</sup> transport cannot be made. The acquisition of an amiloride-sensitive  $\Delta V_i$  that correlates in time with changes in the polarity and magnitude of the  $\Delta V_i$  is suggestive of a developmental change in ionic transport mechanisms. Future studies will attempt to establish the relationship between the Na<sup>+</sup> transport process described here and the ouabain sensitive Na transport system located on the juxtacoele surface of the rabbit trophoctoderm (J.D.B., R.M.B. and Lechene, C. P. unpublished).

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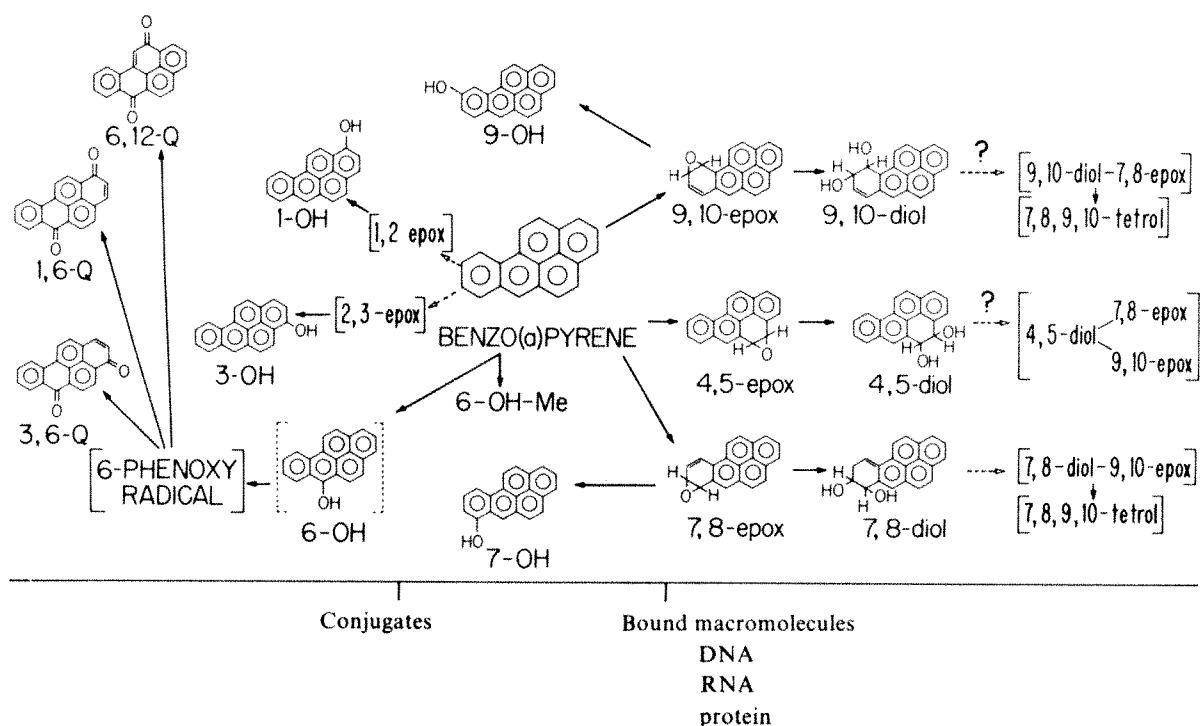
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- Biggers, J. D. & Borland, R. M. *A. Rev. Physiol.* **38**, 95–119 (1976).
- Borland, R. M. in *Development in Mammals* 1 (ed. Johnson, M. H.) (Elsevier-North Holland, Amsterdam, 1977).
- Biggers, J. D., Borland, R. M. & Powers, R. D. *Ciba Fdn Symp.* **52**, 129–153 (1977).
- Gardner, R. L. & Papaioannou, V. E. in *The Early Development of Mammals* (eds Bails, M. & Wild, A. E.) (Cambridge (1975)).
- Daniel, J. C. *Am. Nat.* **98**, 85–98 (1964).
- Schultz, S. G., Frizzel, R. A. & Nellans, H. N. *A. Rev. Physiol.* **36**, 51–91 (1974).
- Powers, R. D. & Tupper, J. T. *Dev. Biol.* **38**, 320–331 (1974).
- Borland, R. M., Biggers, J. D. & Lechene, C. P. *Dev. Biol.* **50**, 201–211 (1975).
- Van Blerkom, J. & Manes, C. *Dev. Biol.* **40**, 40–51 (1974).
- Van Blerkom, J., Manes, C. & Daniel, J. C. *Dev. Biol.* **35**, 262–282 (1973).
- Cross, M. H. & Brinster, R. L. *Expt Cell Res.* **58**, 125–127 (1969); **62**, 303–309 (1970).
- Dörge, A. & Nagel, W. *Pflügers Arch. ges. Physiol.* **321**, 91–101 (1970).
- Salako, L. A. & Smith, A. J. *Br. J. Pharmacol.* **38**, 702–718 (1970).
- Ussing, H. H., Erlj, D. & Lassen, U. *A. Rev. Physiol.* **36**, 17–49 (1974).
- Cross, M. H. *Biol. Reprod.* **8**, 566–575 (1973).
- Ducibella, T., Albertini, D. F., Anderson, E. & Biggers, J. D. *Dev. Biol.* **45**, 231–250 (1975).
- Hastings, R. A. & Enders, A. C. *Anat. Rec.* **181**, 17–34 (1975).

## Divergence of metabolic activation systems for short-term mutagenesis assays

POLYCYCLIC hydrocarbons such as benzo(a)pyrene (BP) are prevalent environmental contaminants<sup>3</sup> and are increasingly suspect as human carcinogens. They are routinely used as tumour agents in laboratory animals<sup>1</sup> and in tissue culture assays<sup>2</sup>. These compounds are produced by the incomplete combustion of carbonaceous material such as fossil fuels used for transportation and industrial energy production. They are biologically inactive as parent compounds and require metabolic activation in tissue to produce a tumorigenically active species of the molecule. Activation is accomplished through the drug metabolising mono-oxygenase enzymes containing cytochrome P450 (refs 4–6), and activate the parent carcinogen to an intermediate epoxide<sup>7,8</sup> which readily alkylates cellular macromolecules.

Figure 1 shows a compilation of known BP metabolites and those in brackets which have not yet been isolated or sufficiently characterised. Epoxide intermediates (4,5-; 7,8-; 9,10-) are further metabolised by microsomal epoxide hydrolases to produce diol derivatives or undergo non-enzymatic ring opening to produce phenols which are labile and form quinones as oxidation products. The metabolite profiles for benzo(a)pyrene in numerous species and tissues such as skin has not produced unique metabolites that are not present in resistant tissues. Quinones are formed both enzymatically and non-enzymatically, and their role in tumorigenesis remains uncertain. Therefore, susceptibility to tumorigenesis by these chemicals may be a function of metabolite kinetics since the rate of formation and removal of the activated carcinogenic species of benzo(a)pyrene may greatly influence the probability of the active molecules alkylating target site for carcinogenesis. There are at least three major enzymatic steps in this biochemical scheme—epoxidase, hydrolase and transferase to water soluble conjugates. Also there is considerable species variation within at least the first enzymatic reaction step<sup>9</sup>. Sims *et al.*<sup>10</sup> showed that the 7,8-diol metabolite was remetabolised by the microsomal oxygenases to a 7,8-diol-9,10-epoxide, and this



**Fig. 1** Benzo(*a*)pyrene metabolites. Drawn structures represent isolated and characterised derivatives. 7,8-epox- and 9,10-epox- have been confirmed through isolation of their respective diols. Bracketed structures are possible metabolites. The 6-phenoxy radical is not stable, but readily seen by its electron-spin resonance signal<sup>23</sup>.

- 6,12-Q, benzo(a)pyrene-6,12-dione  
1,6-Q, benzo(a)pyrene-1,6-dione  
3,6-Q, benzo(a)pyrene-3,6-dione  
1-OH, 1-hydroxy-benzo(a)pyrene

- 3-OH, 3-hydroxy-benzo(*a*)pyrene  
6-OH, 6-hydroxy-benzo(*a*)pyrene  
7-OH, 7-hydroxy-benzo(*a*)pyrene  
9-OH, 9-hydroxy-benzo(*a*)pyrene  
6-OH-Me, 6-hydroxymethyl-benzo(*a*)pyrene  
9,10-epox., benzo(*a*)pyrene-9,10-oxide  
4,5-epox., benzo(*a*)pyrene-4,5-oxide  
7,8-epox., benzo(*a*)pyrene-7,8-oxide  
9,10-diol, 9,10-dihydrodihydroxy-benzo(*a*)pyrene  
4,5-diol, 4,5-dihydrodihydroxy-benzo(*a*)pyrene  
7,8-diol, 7,8-dihydrodihydroxy-benzo(*a*)pyrene

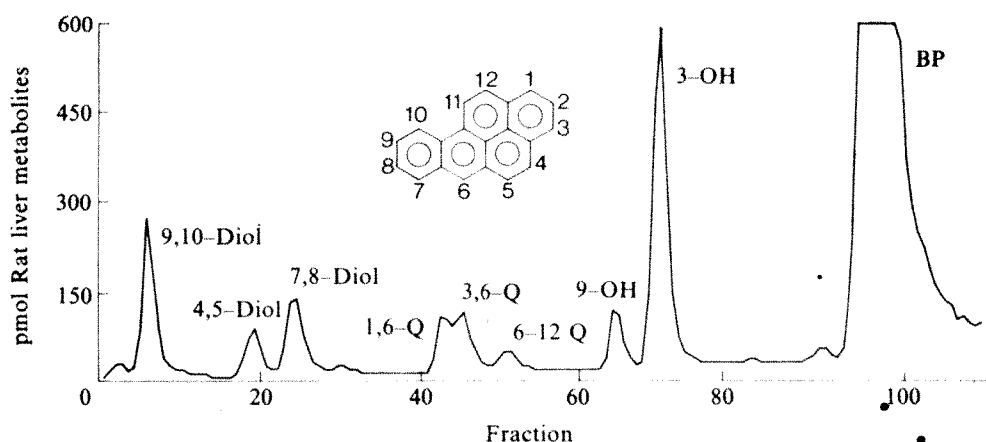
intermediate seems to be the major DNA binding metabolite along the pathway of benzo(a)pyrene metabolism<sup>11-13</sup>.

If the diol-epoxide intermediate is one of the necessary requisites for development of the malignantly transformed state, then it will be critically important to compare metabolite patterns in susceptible and resistant tissues, species and cells. This may help to explain the variance in biological susceptibility to polycyclic hydrocarbons. As there is a marked quantitative variation between liver microsomal metabolism of rat, mouse and hamster<sup>14</sup> there is the strong suggestion that short-term *in vitro* mutagenesis assays either bacterial<sup>15</sup> or mammalian cell<sup>16</sup> which routinely add rat liver microsomal preparation for carcinogen activation may be producing mutagenesis data not

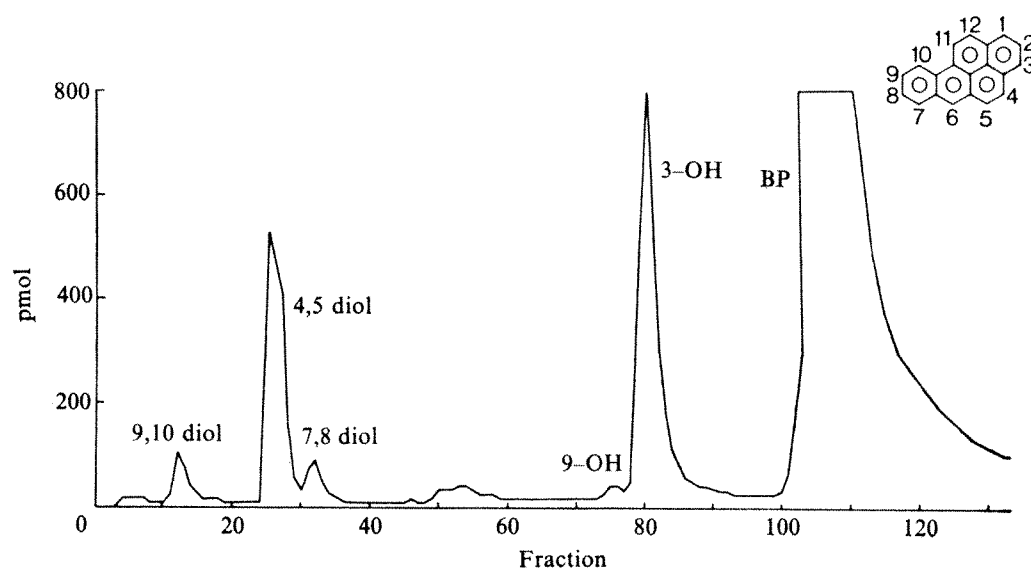
extrapolatable to other species, including man. To test this hypothesis, we have compared benzo(a)pyrene metabolism by liver microsomes, both rat and hamster, against metabolism by hamster embryo cells and hamster embryo cell microsomes with the use of high-pressure liquid chromatography.

To determine the uniformity of the metabolic pathway liver microsomes were prepared from male Sprague-Dawley rats weighing 125–150 g and Golden Syrian hamsters (Charles River, Boston, MA) as previously described<sup>17</sup>. Hepatic monooxygenases were induced by i.p. injection of 5 mg of methylcholanthrene in 0.5 ml of corn oil 40 h before killing. Primary cell cultures were prepared from hamster embryos<sup>14</sup> and secondary cultures ( $8\text{--}10 \times 10^6$  cells

**Fig. 2** Rat liver microsomal metabolism of benzo(*a*)pyrene. High-pressure liquid chromatography. Metabolite separation was performed with a Chromatronics 3500 high-pressure liquid chromatograph (HPLC) fitted with a DuPont 1-m ODS Permaphase column. Elution was by reverse phase, using a methanol-water gradient and  $^{14}\text{C}$ -derivatives as internal standards as previously described<sup>12</sup>. Column pressure was 500 pounds per square inch and oven temperature was 50 °C. Effluent was monitored through an 8  $\mu\text{l}$  flow cell on a DuPont 835 multiwavelength UV-fluorescence spectrophotometer with





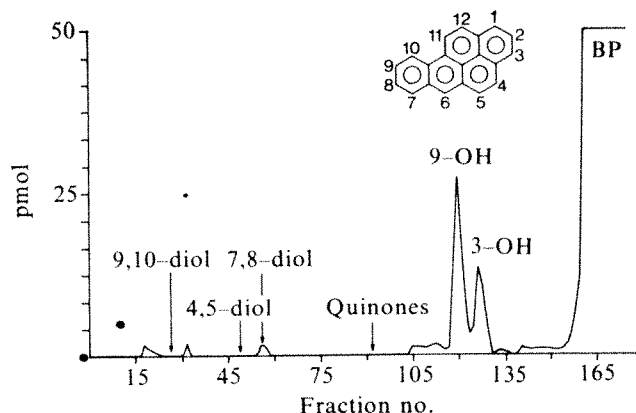


**Fig. 3** Hamster liver microsomal metabolism of  $^3\text{H}$ -benzo(a)pyrene. HPLC analysis of ethyl acetate extractable metabolites. Abbreviations as in Fig. 1 legend.

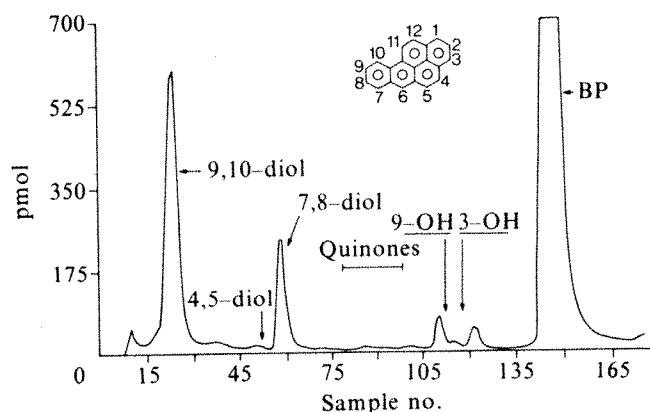
per 100 mm dish  $\times$  10 dishes) were incubated with  $^3\text{H}$ -benzo(a)pyrene for 24 h and the cells and medium extracted into ethyl acetate for metabolite analysis. The microsomal cell pellet (100,000g) was prepared by scraping the cells from the dishes and gently lysing in a Potter-Elvehjem homogeniser in Tris-Cl buffer pH 7.5. Microsomes were suspended in Tris-sucrose buffer pH 7.5 and incubated for 30 min at 37 °C. The incubation contained in 1 ml: 15  $\mu\text{mol}$  Tris-chloride buffer pH 7.5; 100 nmol  $^3\text{H}$ -benzo(a)pyrene (specific activity 200 mCi mmol $^{-1}$ ) 0.35  $\mu\text{mol}$  NADPH; 3  $\mu\text{mol}$   $\text{MgCl}_2$ , and 100  $\mu\text{g}$  protein. Metabolism was halted by the addition of 1.0 ml acetone and the organic soluble metabolites from 10 incubations extracted into ethyl acetate for analysis by HPLC as previously described<sup>18</sup>.

Figures 2-5 clearly show marked differences in the hydroxylated products formed between rodent liver microsomes and intact or lysed cells. This profile variance suggests the monooxygenase system shows perturbation in the metabolic attack on different regions of the molecule. Rat (Fig. 2) and hamster liver (Fig. 3) microsomes show metabolite patterns containing three diols (9,10-; 4,5-; 7,8-dihydro-dihydroxy-BP) three quinones (1,6'- 3,6' 6,12-BP quinone) and two phenols (9-OH; 3-OH-BP) using this chromatography system. Liver contains the highest level of drug metabolising enzymes but is not a major target-site for polycyclic carcinogens. Hamster liver produces almost exclusively 4,5-diol while it forms a lesser component of rat liver metabolism.

**Fig. 4** Metabolism of  $^3\text{H}$ -benzo(a)pyrene by hamster embryo cell microsomes. HPLC analysis of ethyl acetate extractable metabolites. Abbreviations as in Fig. 1 legend.



In contrast, microsomes from hamster cells (Fig. 4) either produce insignificant amounts or further metabolise diols and quinones while forming 9-hydroxy as the major metabolites with less amounts of 3-hydroxy. Conversely, BP metabolism in intact hamster cells (Fig. 5) reverses the metabolite pattern. The 9,10- and 7,8-diols are the major components with only trace amounts of phenols. It would seem intact cells produce predominantly precursor diols for formation of the highly reactive diol-epoxides. Disruption of the cells clearly alter metabolite ratios indicating a requirement for spatial orientation of the enzyme complex within the microsomal membrane. Also, intact cells contain the cytoplasmic conjugates that are important for removal of reactive tumorigenic and toxic intermediates to water



**Fig. 5** Intact hamster embryo cell metabolism of  $^3\text{H}$ -benzo(a)pyrene. HPLC analysis of ethyl acetate extractable metabolites. Abbreviations as in Fig. 1 legend.

soluble excretion products<sup>19,20</sup>. The conjugating enzymes become diluted or lost during cell disruption and microsome preparation.

If activation of specific regions of the carcinogen molecule is more important for tumorigenesis than other regions of the molecule, then it is important that monooxygenase enzymes added to short-term *in vitro* mutagenesis assays reflect as closely as possible the enzyme activities in an *in vivo* situation. False positives or negatives which occur in bacterial assays may be simply a function of using a mammalian activation system to produce changes in bacterial biochemistry. *In vitro* assays utilising intact cell-feeder layers as activation systems for producing reactive

carcinogens for the test cell cultures seem to be the closest for *in vivo* comparisons<sup>17,18</sup>.

Species resistance and susceptibility may be determined by which activation pathway is followed. The route, once selected, may in turn decide the kinetics of formation and disappearance of the activated carcinogenic species of the molecule and dictate whether that given species is a significant risk to polycyclic hydrocarbon induced tumorigenesis. Research supported by the National Cancer Institute (Yol-CP-50200) and the Department of Energy under contract with Union Carbide Corporation.

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1. *Publ. publ. Hlth Serv., Wash.* No. 149 (1973).
2. Heidelberger, C. *Ann. Review Biochem.* **44**, 79 (1975).
3. Committee on Biologic Effects of Atmospheric Pollutants. (National Academy of Sciences, Washington, D.C., 1972).
4. Gelboin, H. V. *Cancer Res.* **10**, 1 (1967).
5. Conney, A. H. *Pharmacol. Rev.* **19**, 317 (1967).
6. Hebert, D. W., Robinson, J. R., Niwa, A., Kumaki, K. & Poland, A. P. *J. Cell Physiol.* **85**, 393 (1975).
7. Selkirk, J. K., Croy, R. G. & Gelboin, H. V. *Arch. biochem. Biophys.* **168**, 322 (1975).
8. Sims, P. & Grover, P. L. *Adv. Cancer Res.* **20**, 165 (1974).
9. Haugen, D. A., Coon, M. J. & Nebert, D. W. *J. biol. Chem.* **251**, 1817 (1976).
10. Sims, P., Grover, P. L., Swaisland, A., Pal, K. & Hewer, A. *Nature* **252**, 326 (1974).
11. Weinstein, I. B., Jeffrey, A. M., Hennessee, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H. & Nakamishi, K. *Science* **193**, 592 (1976).
12. Yang, S. K., McCourt, D., Roller, P. P. & Gelboin, H. V. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2494 (1976).
13. King, H. W. S., Osborne, M. R., Beland, F. A., Harvey, R. G. & Brookes, P. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2679 (1976).
14. Selkirk, J. K., Croy, R. G., Wiebel, F. J. & Gelboin, H. V. *Cancer Res.* **35**, 4476 (1976).
15. Ames, B. N., McCann, J. & Yamasaki, E. *Mutat. Res.* **31**, 347 (1975).
16. Kuroki, T., Drevon, C. & Montesano, R. *Cancer Res.* **37**, 1044 (1977).
17. Kinoshita, N., Shears, B. & Gelboin, H. V. *Cancer Res.* **33**, 1936 (1973).
18. Selkirk, J. K., Croy, R. G. & Gelboin, H. V. *Science* **184**, 169 (1974).
19. Baird, W. M., Chern, C. J. & Diamond, L. *Cancer Res.* **37**, 3190 (1977).
20. Cohen, G. M. & Moore, B. P. *Biochem. Pharmacol.* **25**, 1623 (1976).
21. Huberman, E. & Sachs, L. *Int. J. Cancer* **13**, 326 (1974).
22. Huberman, E. & Sachs, L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 188 (1976).
23. Nagata, C., Tagashira, Y. & Kodama, M. in *Chemical Carcinogenesis* (eds P. O. P. Ts'o & J. A. DiPaolo), ch. 2, 87-111 (New York, Dekker, 1974).

## Identification of non-proliferating cells in melanoma B16 tumour

THE detection of growth fraction and the characterisation of non-proliferating cells are extremely important in human tumour therapy<sup>1,2</sup>. Cytological criteria for individual objective discrimination *in vivo* between non-proliferating and proliferating cells with the same DNA content have not been previously available<sup>3</sup>. It has been shown recently, *in vitro*, that the transition from the non-proliferating to proliferating state (G0-G1) of WI-38 cells—human diploid fibroblasts—can be objectively characterised in the intact cell by flow microfluorimetry<sup>4</sup> and geometric-densitometric texture analysis<sup>5</sup>. Similar differences in nuclear morphometry and chromatin conformation between proliferating G1 and non-proliferating 'G0+Q' cells are reported here for the first time for tumour cells *in vivo*, by parallel utilisation of objective image analysis and multiparameter laser flow microfluorimetry, both correlated with autoradiography.

The experimental details have been published elsewhere<sup>4-6</sup>. Basically, the B16 melanoma tumour was grown in the foot pad of C57 mice: a suspension of  $3 \times 10^5$  tumour cells was grafted s.c. into the foot pad of a 3-month-old mouse and 10 d later the tumour was excised. Triplicate smear preparations of tumour tissue were Feulgen-stained, using 1 M HCl at 60 °C for 10 min<sup>7</sup>, and image analysis of the microscopic slides was performed using a Quantimet 720-D automated image analyser (Cambridge Corp, New York).

The following parameters were measured, as previously described<sup>3,6</sup>, for each nuclear image: integrated optical

density (IOD, proportional to DNA content), area, perimeter, projection, form factor (area divided by the square of the perimeter), average optical density (IOD divided by area), and mean bound path (area divided by projection).

Figure 1 shows a two-dimensional plot of about 100 tumour nuclei. The ordinate corresponds to the integrated optical density while the abscissa gives the average optical density ( $A_{av}$ ). The IOD histogram reveals a distribution typical of a logarithmically growing cell population with a large distinct peak, resembling cells with the same DNA content (G0+G1). On the abscissa the  $A_{av}$  histogram of

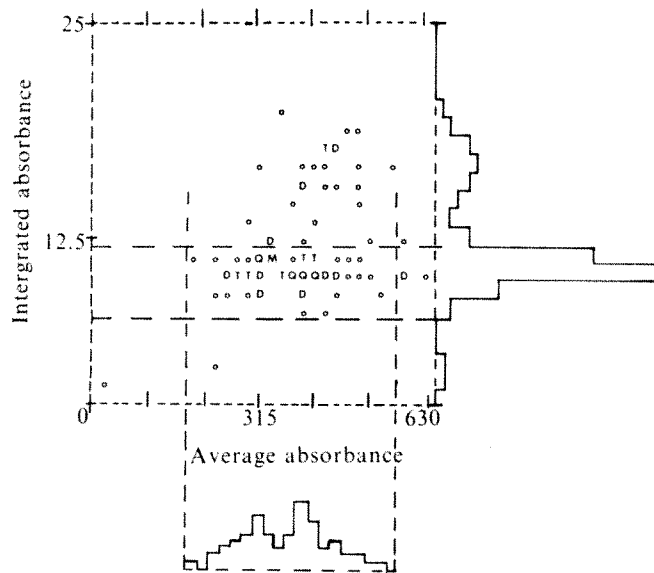
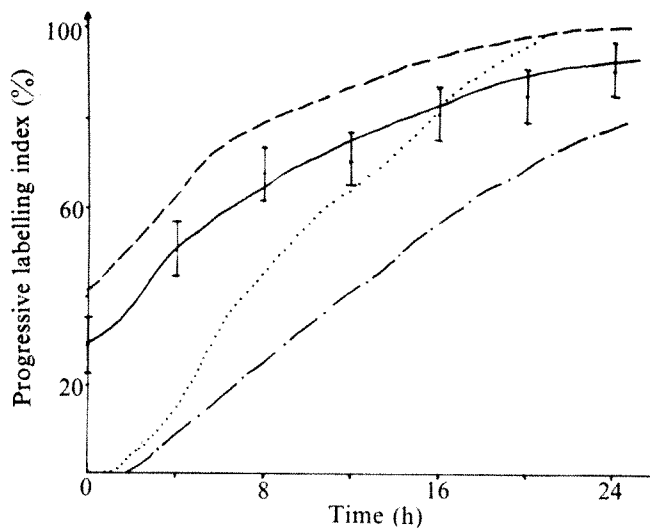


Fig. 1 Scattergram of Feulgen-stained melanoma B16 nuclei in a smear preparation. The integrated optical density of each nucleus is presented over its average optical density (both in arb. units). + = 1 cell, D = 2 cells, T = 3 cells, Q = 4, M = 5 or larger. The rectangle bound by the dashed lines containing the G0+Q and G1 cells. Fibroblasts contamination in our tumour cell suspension is less than 3%. The raw data were processed by various programs, loaded on a PDP11/40 computer<sup>6</sup>, for screening, purification, necessary statistics and computer display, as shown. Absorbance = optical density.

the same cells, present in the IOD unimodal peak, shows instead a bimodal distribution with two clearly separated peaks. By a striking analogy with our previous findings *in vitro* using WI-38 cells<sup>4,5</sup> (the chromatin in the G1 cells is more dispersed than in G0 confluent cells) we may suppose that the two peaks correspond respectively to the proliferating G1 (dispersed chromatin) and non-proliferating G0 (condensed chromatin) tumour cells. This would result in a growth fraction (number of all proliferating cells/total number of cells) of about 0.65. Without any further direct proof, however, our identification of 'G0+Q' and G1 cells, as inferred from the clustering of the data in two subpopulations and the analogy with WI-388, could remain open to criticism. Therefore we conducted a continuous labelling experiment (Fig. 2) in order to achieve an independent estimate of the growth fraction using autoradiographic techniques.

One must be careful, however, in analysing progressive labelling data. Because the tumour is in balanced exponential growth, the proliferating and non-proliferating compartments are in a dynamic equilibrium. Therefore some proliferating P cells must subsequently become non-proliferating (G0+Q) and some G0 cells may resume proliferation. Also, some Q cells will become necrotic and disappear from the system. Thus, in order to determine the growth fraction from progressive labelling data, we must make a dynamic model<sup>7</sup> (see Fig. 2), which best fits the



**Fig. 2** Progressive labelling index of B16 melanoma cells. The curves show the predicted progressive labelling index of the B16 melanoma by a dynamic model<sup>7</sup>, which includes a proliferating P compartment, a reversible resting G0 compartment and an irreversible non-proliferating Q compartment. We have found that the model is fairly insensitive to the specific fraction of cells in G0 and Q and the continuous labelling curve depends only on the total (G0+Q) non-proliferating compartment. The solid line shows the predicted labelling index of the entire population. Superimposed are the experimental data points (●), with error bars. The dashed line shows the predicted labelling of the proliferating P compartment. The dotted-dashed line shows the labelling of the (G0+Q) non-proliferating compartment. The dotted line shows the fraction of the G1 compartment labelled. Note that there is not a great difference between the labelling of the (G0+Q) and G1 cells. This is a consequence of the balanced growth assumption. The experimental labelling indices (means and standard deviation), were determined by autoradiography of melanoma B16 tumours growing in C-57 black mice, at various hourly intervals after a single injection of <sup>3</sup>H-thymidine (0.25  $\mu$ Ci g<sup>-1</sup> body weight), followed, at 4-h intervals, by six injections of <sup>3</sup>H-thymidine (0.25  $\mu$ Ci g<sup>-1</sup> each). To determine the growth fraction, we fit a mathematical model<sup>7</sup> to the data, by using as input the cell cycle phases residence times, doubling time (45.9  $\pm$  1.5 h) and flash labelling index (0.27); by iteration, the predicted labelling index which best fitted the continuous labelling data are consistent with Q cells lost of 0.07 per h and a growth fraction of 0.68  $\pm$  0.03.

data with a (G0+Q = NP) non-proliferating compartment of about 32%. Since only cells in the proliferating P compartment may divide, at every division a constant fraction of P cells become NP cells. Because all P cells will eventually be labelled, this implies that cells in the non-proliferating compartment must eventually be labelled (Fig. 2), making it impossible to have direct evidence that all cells out of cycle by image analysis (Fig. 1) are also cells out of cycle by autoradiography, that is, unlabelled. Therefore, to make our discrimination of 'G0+Q' from 'G1' cells more than circumstantial, we analyse a cell suspension from the melanoma tumour by an independent method—multiparameter laser flow microfluorimetry<sup>4,9</sup>. Unfixed cells were stained with acridine orange (AO), either directly as previously described<sup>4,10</sup>, or after treatment with Triton and chelating agents<sup>9</sup>. Both procedures yield similar results (Fig. 3), showing either in the green<sup>4</sup> or red emission<sup>9</sup> a peak with lower fluorescence clearly separated from fluorescence distribution typical of log-phase population (that is, proliferating cells in G1, S, G2 and M phases).

Analysis of the two larger peaks, with different fluorescence, but with similar low angle scatter (size), sorted by a two-parameter fluorescence activated cell sorter, shows that, in our experimental conditions, both peaks contain only single tumour cells, with the same amount of DNA (diploid), undistinguishable by visual observation. Furthermore, 8 h after progressive labelling with <sup>3</sup>H-thymidine, the

tumour cells with lower fluorescence show less incorporation of radioactive thymidine (non-proliferating compartment) than tumour cells with larger fluorescence ('G1'), compatible with the prediction of the model outlined in Fig. 2. For 12 different experiments, the fractions of non-proliferating tumour cells, as determined by a computer fitting to the experimental data<sup>10</sup>, range between 29% and 35% (Fig. 3 and Table 1). The agreement among autoradiography, laser flow microfluorimetry and image analysis in determining the fraction of non-proliferating cells in the melanoma tumour *in vivo*, is striking (Table 1).

**Table 1** Percentage of cells in each cycle phase for the same melanoma B16 tumour cell population growing in C-57 black mice

	G0+Q	G1	S	G2+M
Autoradiography	32 $\pm$ 3	28 $\pm$ 10	27 $\pm$ 7	13 $\pm$ 4
Automated image analysis	34 $\pm$ 3	34 $\pm$ 2	24 $\pm$ 4	9 $\pm$ 1
Laser flow microfluorimetry	32 $\pm$ 3	35 $\pm$ 4	23 $\pm$ 4	10 $\pm$ 2

Determined by autoradiography, laser flow microfluorimetry and image analysis. The means and standard deviations have been obtained from up to 12 different experiments. Experimental details are given in the text.

These independent autoradiographic and FMF studies permit the identification, as 'G0+Q' and 'G1' compartments, of the two peaks in  $A_{av}$  distribution of melanoma cells with the same IOD (DNA) (Fig. 1), which corresponds to the two peaks in green fluorescence distribution of the same melanoma cell with similar size and diploid levels (Fig. 3). Specifically, lower  $A_{av}$  of the nuclear chromatin corresponds to higher binding sites for acridine orange in intact cells ('G1' cell), while higher  $A_{av}$  corresponds to lower binding sites ('G0+Q' cell). Calculating mean values of the optical parameters for each of the two cell subpopulations respectively in the 'G0+Q' and in the 'G1' peak, we get the results presented in Table 2. While the two tumour cell populations have the same DNA content, the average optical density and the form factor of the G1 cells are significantly ( $P < 0.01$ ) lower than the respective values of the (G0+Q) cells. The increase in average horizontal projection and the resulting decrease in mean bound path (Table 2) in the face of both increasing area and perimeter, strongly suggest that the decrease in two-dimensional circular geometry (smaller form factor) is partially due to a significant increase in surface convolution—merely a change in shape to a regular but less circular geometry<sup>5,6</sup>. Thus, 'G1' nuclei expose a bigger (with respect to 'G0+Q' cells) surface to intercalating dyes than to active enzymes, such as RNA polymerase, compatible with the frequently reported increase in template activity during G0-G1 transition of

**Table 2** Comparison of integrated optical density (DNA content), mean bound path, average optical density and form factor of 'G0+Q' and G1 melanoma B16 tumour cells

	(G0+Q) cells	G1 cells
Integrated optical density (arb. units)	10,720 $\pm$ 1,270	10,800 $\pm$ 1,300
Average optical density (arb. units)	438 $\pm$ 67	287 $\pm$ 40
Form Factor (Undimensional)	(5.6 $\pm$ 1.2) $10^{-2}$	(3.2 $\pm$ 1.4) $10^{-2}$
Mean bound path ( $\mu$ m)	4.06 $\pm$ 0.4	3.16 $\pm$ 0.4
Horizontal projection ( $\mu$ m)	6.3 $\pm$ 1.2	11.6 $\pm$ 3.1

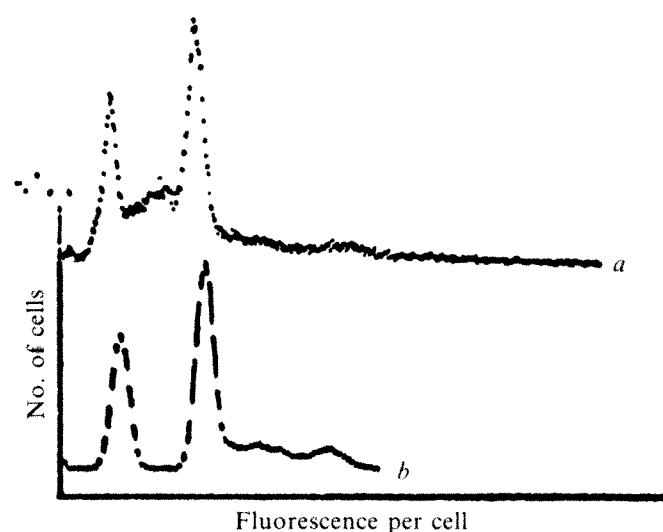
Cell assignment is obtained in the bimodal distribution of average optical density ( $A_{av}$ ) of tumour cells with the same DNA content (IOD), but with  $A_{av}$  larger (G0+Q) and smaller (G1) than 360 arbitrary units (values are means  $\pm$   $\sigma$ ).



different cell lines<sup>8</sup>. Furthermore, the scatter plot of form factor against average optical density (not shown) presents no overlap between 'G0+Q' and G1 tumour cells, that is, the same cell which presents a lower degree of chromatin condensation (Fig. 1, Table 2) shows also an increase of chromatin convolution (Table 2). A convincing and striking biological implication is that these phenomena seem to occur whenever a cell increases the metabolic activity of its DNA, during early G1<sup>3,6</sup>, G1-S<sup>3,6</sup>, and G0-G1<sup>3,8</sup>.

Our results prove, therefore, that geometric and densitometric image analysis, as supported by multiparameter laser flow microfluorimetry, provide a simple optical method for objective individual identification of proliferating G1 and non-proliferating 'G0+Q' cells, with the same DNA content, in an *in vivo* system, such as melanoma B16 tumour growing in mice (Figs 1-3, Tables 1-2). The differences in the nuclear morphometry can be interpreted in terms of increased chromatin dispersion and convolution in proliferating G1 cells<sup>3,3</sup>, compatible both with the increased dye uptake<sup>6,9</sup> (caused also by a larger amount of RNA in G1 cells) and with the changes in template activity and tertiary-quaternary structure observed in *in vitro* systems<sup>8,11</sup>.

Finally, the possibility of determining, by these direct and fast means, the growth fraction<sup>1-3</sup> in intact tumour cell suspensions, *in vivo*, could become extremely useful in



**Fig. 3** Frequency distribution of green fluorescence per cell from a suspension of melanoma B16 tumour cells, obtained after 20 min of trypsinisation in 0.25% trypsin solution. Unfixed cells were stained directly<sup>4</sup> with acridine orange at  $R$  ( $\mu\text{M}$  acridine orange per  $\mu\text{M}$  DNA) = 1.0; here the green emission was measured in order to monitor differences in chromatin DNA binding sites (which are known to be somewhat smaller in non-proliferating cells<sup>3,4,8</sup>). Similar distributions were obtained after treatment with non-ionic detergent at pH 3.0 and exposure to chelating agents<sup>9</sup>: unfixed cells were stained with acridine orange<sup>9</sup> and their total (or red) emission measured in order to take advantage also of differences in RNA<sup>9</sup> (at substantially smaller levels in non-proliferating cells<sup>6,9</sup>). In both instances, a simultaneous on line acquisition and reduction of low angle scatter and fluorescence was necessary in order to achieve discrimination (by selecting cells with larger scatter) of viable melanoma cells from necrotic cells and lymphocytes, which infiltrate the tumour. The experimental distribution (a) was obtained on a B-D fluorescence activated cell sorter on line with a PDP11/40, triggering on homogeneous cell populations of larger size (forward angle scatter). The best theoretical<sup>10</sup> fit (b) to the data shown above gave a non-proliferating compartment G0+Q of 31%, with the proliferating compartment divided among G1 (36%), S (23%), and G2+M (10%) phases. The increased dye uptake for G1 tumour cells in respect to G0+Q is identical (about 2.45 times larger) to that previously reported for G0-G1 transition, *in vitro*<sup>3,4</sup>. Fluorescence and low angle forward light scattering were measured either on a Cytofluorograf 4800A (Ortho Systems Co.) or a FACS-I Cell Sorter (Beckton-Dickenson), both on line with a PDP11/40 digital computer (up to one million cells were measured).

human cancer chemotherapy, besides providing objective evidence for the existence of a different degree of chromatin superpacking<sup>4,5,8,11</sup> for cells in the non-proliferating compartment, even in animal tumour systems. The sorting of homogenous 'G0+Q' and 'G1' tumour cells, will allow also further characterisation of the molecular mechanism controlling mammalian cells proliferation *in vivo*.

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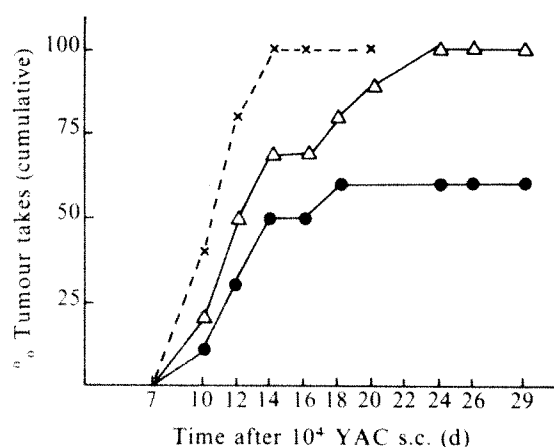
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1. Mendelsohn, M. *J. natn. Cancer Inst.* **28**, 1015-1027 (1962).
2. Skipper, H. H. *J. Am. med. Ass.* **230**, 1033-1035 (1974).
3. Nicolini, C. *Biophys. biochem. Acta* **458**, 243-282 (1976).
4. Nicolini, C., Kendall, F., Desai, C., Clarkson, B. & Fried, J. *Expl Cell Res.* **106**, 111-118 (1977).
5. Nicolini, C., Giaretti, W., Desai, C. & Kendall, F. *Expl Cell Res.* **106**, 119-125 (1977).
6. Kendall, F., Swenson, R., Borun, T., Rowinski, J. & Nicolini, C. *Science* **196**, 1106-1108 (1977).
7. Fried, J. *et al. Comput. Biomed. Res.* **7**, 333-359 (1974).
8. Baserga, R. & Nicolini, C. *Biophys. biochem. Acta* **458**, 109-134 (1976).
9. Tragano, F., Darzynkiewicz, Z., Sharpless, T. & Melamed, M. *J. histochem. Cytochem.* **25**, 46-56 (1977).
10. Wu, S., Zietz, S., Kendall, F., Toton, S. & Nicolini, C. *Proc. 3rd Symp. Pulse Cytophotometry* (1977).
11. Nicolini, C. & Kendall, F. *Physiol. chem. Phys.* **29**, 3 (1977).

## Role of non-conventional natural killer cells in resistance against syngeneic tumour cells *in vivo*

IMMUNE reactions of 'conventional' T and B lymphocytes are generally thought to constitute major parts of any measurable, 'specific' tumour resistance against autologous tumours. There is no doubt that protective immunity can be induced against subsequently transplanted syngeneic tumours in experimental systems<sup>1</sup>. But, there is only scanty evidence to suggest that conventional immune reactions can provide resistance against such tumour cells when transplantation is made into normal individuals. Yet, it is frequently found that even long-transplanted syngeneic tumour cells may require comparatively high numbers of cells to ensure tumour take in normal recipients. Mice failing to succumb to small numbers of tumour cells can frequently be shown to reject a second graft of the same tumour with similar vigour<sup>2</sup>. Thus, natural resistance against tumours may occur with no display of classical, immunological memory. That such natural protective forces do exist against tumours has been claimed for many years<sup>3</sup>, but its underlying basis is poorly understood. Here, we present data indicating that naturally occurring killer cells may play a decisive part in providing resistance against syngeneic tumour cells *in vivo*.

Natural killer (NK) cells with the ability to kill several kinds of tumour cells in short-term *in vitro* assays are found in mice and in other species (see refs 4 and 5 and refs therein). These NK cells are most numerous in spleen and blood and are under autonomous control by stem cells in the bone marrow<sup>6</sup>; they develop in the absence of the thymus<sup>7,8</sup> and do not (by various criteria) belong to either conventional B or T lymphocytes or the macrophage-monocytic lineage. They appear and disappear in a highly characteristic manner according to the age of the mouse<sup>7,8</sup>. The level of NK activity in individual animals is under comparatively strict genetic control<sup>9</sup>, allowing the classification of inbred mouse strains into 'low' or 'high' NK type. It is therefore possible,



**Fig. 1** Percentage tumour takes in animals inoculated with  $10^4$  YAC tumour cells subcutaneously. ●, (A/Sn  $\times$  C57BL/6) $F_1$  mice irradiated with 750 R and reconstituted with (A/Sn  $\times$  C57BL/6) $F_1$  anti-Thy serum + C treated bone marrow cells. △, (A/Sn  $\times$  C57BL/6) $F_1$  mice irradiated with 750 R and reconstituted with (A/Sn  $\times$  A.BY) $F_1$  anti-Thy serum + C treated bone marrow cells. ×, A/Sn control mice. 10 animals in first two groups, 6 in the last one. All animals with tumours died with tumours. For detailed conditions of transfer see ref. 10. A.BY and C57BL/6 mice have the same H-2 = H-2B.  $0.02 < P < 0.05$  in  $\chi^2$  test comparing the two groups of marrow reconstituted  $F_1$  mice. If data are pooled with data from Table 1 the differences in tumour takes observed between NK 'high' and NK 'low'  $F_1$  hybrids are significant at  $P < 0.001$ .

using lethal irradiation followed by reconstitution with histocompatible bone marrow stem cells from low or high NK strains, to produce individual adult mice with distinct variation in NK activity but otherwise almost identical with regard to histocompatibility<sup>10</sup>.

Preliminary evidence suggests that there may be a positive correlation between the levels of NK activity (as measured *in vitro*) in a given individual and the degree of resistance of the same animal towards transplantation with a NK-sensitive tumour<sup>11,12</sup>. Here, we show that tumour resistance *in vivo* is indeed positively correlated with the *in vitro* NK activity using two independent methods of changing NK activity *in vivo*. In the first experiments adult  $F_1$  hybrid mice were irradiated and repopulated with bone marrow cells from high or low NK donors, both being histocompatible with the recipient as well as the tumour cells subsequently to be used. As mentioned above, it is already known that the eventual NK activity of the spleen cells of such repopulated animals is predetermined at the level of the bone marrow donor. To exclude *in vitro* artefacts being transferred into *in vivo* experiments, in rejection experiments we used the *in vivo* line of the YAC tumour line. YAC is a Moloney lymphoma of A/Sn genotype, known to be highly sensitive to NK cells when grown as an *in vitro* line<sup>13</sup>. The *in vivo* line which has never grown *in vitro* is also sensitive to NK activity although at a significantly lower

level. Figure 1 shows that A/Sn  $F_1$  hybrids reconstituted so as to become either low or high with regard to NK activity express significant differences in resistance to YAC tumour cells. Thus, 'high' NK  $F_1$  hybrids display significantly higher resistance than the 'low' hybrids when transplanted with  $10^4$  YAC cells. When the tumour dose was increased to  $10^5$  cells all mice did now succumb to tumour outgrowth with only minor differences in survival time (data not shown).

These results are consistent with the suggestion that NK cells play a decisive part in the rejection of histocompatible tumour cells. But, as these experiments were carried out in animals with irradiated but otherwise intact thymus it could be argued that the observed differences were determined by conventional T lymphocytes reacting against tumour-associated antigens in a 'normal', immune manner. Thus, a new series of experiments was set up but using animals thymectomised at an adult age before irradiation and marrow protection. The success of thymectomy was monitored by immunisation with polyvinylpyrrolidone (PVP), a T-independent antigen as well as with horse erythrocytes, a T-dependent antigen<sup>14</sup> (data not shown). The animals were then transplanted with YAC cells and assessed for *in vivo* resistance to tumour outgrowth. Table 1 shows that thymectomy had no impact either on the level or on the pattern of tumour resistance: high NK marrow donors provided the host with eventual high resistance towards transplantation with YAC cells in the same manner as in the experiments depicted in Fig. 1. Thus, absence of immunocompetent T lymphocytes in the present system has no detectable role in providing *in vivo* resistance against syngeneic tumour cells.

NK activity changes more markedly with the age of the mouse than do B and T lymphocyte functions<sup>7,8</sup>. Thus, NK activity appears abruptly around day 21 after birth and remains high until approximately 3 months of age followed by a rapid drop. Results from one system suggest a change in tumour resistance *in vivo* with age that would seem to parallel the NK activity<sup>12</sup>. We have carried out experiments in our NK system to investigate this matter. Figure 2 shows the results of such an experiment carried out in a 'high' NK A/Sn  $F_1$ -hybrid combination: there is a dramatic drop in resistance between 6 weeks and 6 months of age which corresponds with the reduction of *in vitro* NK activity<sup>7,8</sup>. Experiments carried out in additional combinations using NK-sensitive tumour targets confirmed the observed age dependent pattern of *in vivo* resistance. Thus, these data also support the view that NK cells play a most decisive part in determining *in vivo* resistance against several kinds of syngeneic tumour cells.

As indicated above, results from other workers support our finding<sup>2,12</sup>. Furthermore, NK cells are recruited when NK-sensitive tumour cells are being inoculated<sup>15</sup> and can be isolated from tumour biopsies growing at locations where normally no NK cells would be detected<sup>16</sup>. Thus NK cells are apparently capable of migration to and destruction of sensitive targets in diverse regions of the body. Our findings may also shed some light on the confusing field of nonspecific immunotherapy of tumours.

**Table 1** Intact *in vivo* resistance towards semi-syngeneic tumour cells in T-deficient animals

Expt	Recipient animals*	Bone marrow cells†	NK type‡	Tumour cells§	Frequency of tumour takes		
					Day 9	Day 20	Day 36
1	(A/Sn $\times$ 129/J) $F_1$	(A/Sn $\times$ 129/J) $F_1$	low	YAC, $10^3$ , s.c.	0/8	6/8	7/8
		(A/Sn $\times$ C57BL/6) $F_1$	high	YAC, $10^3$ , s.c.	0/8	0/8	0/8
2	(A/Sn $\times$ C57BL/6) $F_1$	(A/Sn $\times$ 129/J) $F_1$	low	YAC, $5 \times 10^3$ , s.c.	0/15	9/15	9/15
		(A/Sn $\times$ C57BL/6) $F_1$	high	YAC, $5 \times 10^3$ , s.c.	0/14	1/14	1/14

The experiments show a positive correlation between *in vivo* resistance and *in vitro* NK cell activity.

The differences in tumour takes observed comparing NK 'low' with NK 'high'  $F_1$  mice are statistically significant for both experiments ( $P < 0.001$  in  $\chi^2$  tests).

\*Thymectomised at 1 month of age, irradiated with 750 R 24 or 26 d later. Reconstituted with anti-Thy + C treated bone marrow cells in Expt 1, with foetal liver cells in Expt 2 with genotype as indicated. Success of thymectomy was assessed by immunisation with PVP and horse erythrocytes respectively. Tumour cells grafted 3 weeks after reconstitution.

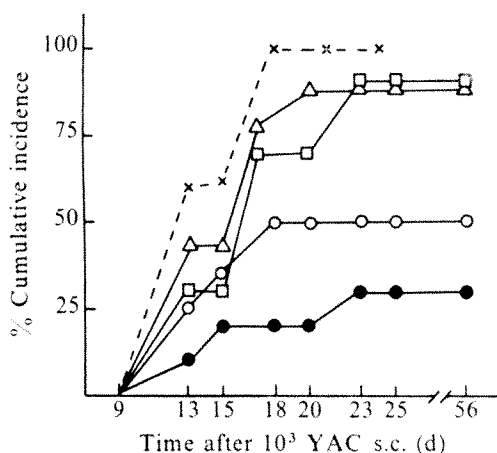
†Genotype of marrow or foetal liver cells. C57BL/6 and 129 have the same H-2 = H-2B.

‡NK activity of reconstituted mice as assessed *in vitro*<sup>13</sup>. (A/Sn  $\times$  129/J)  $F_1$  spleen cells have normally at least 5 times lower NK cell activity compared with (A/Sn  $\times$  C57BL/6) $F_1$  reconstituted mice.

§Transplantation subcutaneously of the H-2A tumour YAC at cell number indicated.

||Animals displaying tumour growth out of total number grafted. All tumour-bearing animals died with tumours.

Thus, two agents (BCG and *Corynebacterium parvum*) which are claimed to sometimes induce significant increases in tumour resistance *in vivo*<sup>17,18</sup> have recently also been found to have grave impacts on NK cell activity<sup>15,19</sup>. Interestingly, either enhancing or strongly suppressive effects on NK cells could be induced by the same agent depending on the conditions used for administration (Ojo, E., Haller, O. & Wigzell, H., unpublished work). It therefore seems possible that parallel measurements of NK activity *in vitro* may allow a controlled use of such immunotherapeutic substances in a manner previously not possible.



**Fig. 2** Percentage tumour takes in (A/Sn x CBA/H) $F_1$  mice of different ages inoculated with  $10^3$  YAC cells subcutaneously. (A/Sn x CBA) $F_1$  is a 'high' NK combination<sup>11</sup>. x, A/Sn control mice, 3 months of age. A/Sn mice are 'low' in NK activity. (A/Sn x CBA) $F_1$  mice: ●, 31/2 weeks of age; ○, 6 weeks of age; □, 6 months of age; Δ, more than 9 months of age. Each group comprised of 8–10 mice. All animals carrying tumours died with tumours.  $0.001 < P < 0.01$  in  $\chi^2$  test comparing the two groups of young mice (6 weeks or younger) with the two groups of old mice (6 months or older). If data from additional experiments (see text) are pooled,  $P < 0.001$ .

Our findings strongly support the suggestion that natural resistance against tumour outgrowth *in vivo* may be exerted largely via cells of a non-conventional type. Thus, a high positive correlation between NK cell activity *in vitro* and tumour resistance levels *in vivo* was found using two distinct methods of analysis. On the other hand, the cells normally considered to be the most important involved in cell-mediated immune reactions, that is, the thymus-dependent lymphocytes, failed to contribute to this *in vivo* resistance. If these results are found to be generally applicable, then this would suggest the existence, outside the conventional immune system, of a surveillance mechanism with a comparatively selective ability to recognise and destroy aberrant malignant cells *in vivo* through direct lytic contact mechanisms.

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- Klein, G. *Harvey Lectures* **69**, 71–102 (1975).
- Greenberg, A. H. & Greene, M. *Nature* **264**, 356–359 (1976).
- Keller, R. in *Immunobiology of the Macrophage* (ed. Nelsson, D. S.) 487–510 (Academic, New York, 1976).
- Kiessling, R. & Haller, O. in *Contemporary Topics in Immunobiology* (ed. Hanna, M. C.) (Plenum, New York, in the press).
- Herberman, R. B. & Holden, H. T. *Adv. Cancer Res.* (eds Klein, G. & Weinhouse, S.) (Academic, New York, in the press).
- Haller, O. & Wigzell, H. *J. Immun.* **118**, 1503–1506 (1977).
- Kiessling, R., Klein, E., Pross, H. & Wigzell, H. *Eur. J. Immun.* **5**, 117–121 (1975).
- Herberman, R., Nunn, M. E. & Lavrin, D. H. *Int. J. Cancer* **16**, 216–229 (1975).
- Petranyi, G. *et al.* *Immunogenetics* **3**, 5–28 (1976).
- Haller, O., Kiessling, R., Örn, A. & Wigzell, H. *J. exp. Med.* **145**, 1411–1416 (1977).
- Kiessling, R., Petranyi, G. G., Klein, G. & Wigzell, H. *Int. J. Cancer* **15**, 933–940 (1975).
- Sendo, F., Aeki, T., Boyse, E. A. & Bufo, C. K. *J. natn. Cancer Inst.* **55**, 603–609 (1975).
- Kiessling, R., Klein, E. & Wigzell, H. *Eur. J. Immun.* **5**, 112–117 (1975).
- Playfair, J. H. L. & Marshall-Clarke, S. *Immunology* **24**, 579–588 (1973).
- Herberman, R. B., Nunn, M. E., Holden, H. T., Staal, S. & Djeau, J. Y. *Int. J. Cancer* **19**, 555–564 (1977).
- Becker, S. & Klein, E. *Eur. J. Immun.* **6**, 892–898 (1976).
- Halpern, B. *et al.* in *Ciba Fdn Symp.* **20**, 217–236 (1973).
- Bast, R. C., Bast, B. S. & Rapp, H. J. *Ann. N. Y. Acad. Sci.* **277**, 60–93 (1976).
- Wolfe, S. A., Tracey, D. E. & Henney, C. S. *Nature* **262**, 584–586 (1976).

## Tumour cell lines induce interferon in human lymphocytes

VIRAL infection of cells induces synthesis and release of interferon which renders uninfected cells resistant to subsequent virus infection. Interferon production can also be stimulated by non-viral agents, such as microorganisms<sup>1</sup>, substances of microbial origin and synthetic polymers<sup>2</sup>. Moreover, interferon can be stimulated in lymphocytes by mitogenic lectins<sup>3</sup>, antilymphocyte sera<sup>4</sup>, viral antigens<sup>5–7</sup>, PPD<sup>8</sup> and exposure to allogeneic cells<sup>9</sup>. An antitumour effect of interferon and interferon inducers has been demonstrated *in vivo*<sup>10–12</sup>. It has been postulated that interferon may act by directly inhibiting tumour cell growth<sup>13</sup> and by stimulating host defence mechanisms<sup>14–16</sup>. We report here that certain human cell lines are able to induce high levels of interferon on contact with human lymphocytes.

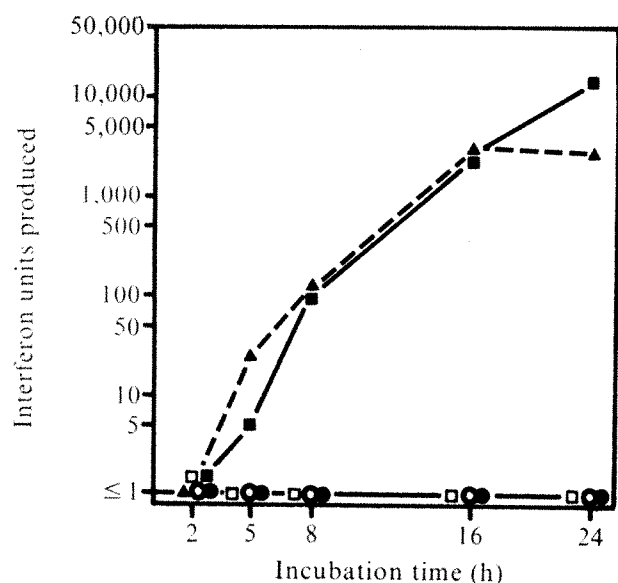
Purified human peripheral blood lymphocytes were cultured with confluent monolayers of various human cell lines. The antiviral activity of the supernatant was measured by inhibition of the cytopathic effect of vesicular stomatitis virus on monolayers of human foetal skin fibroblasts. Eight human tumour-derived cultures and two simian virus 40 (SV40)-transformed cultures induced high levels of antiviral activity when cultured with human lymphocytes (Table 1). The supernatant from cell lines cultured alone or from lymphocytes alone did not show antiviral activity (Fig. 1). All 12 human fibroblast lines derived from normal tissues, 13 out of 15 lines transformed by SV40, and five tumour-derived cell lines, were unable to induce interferon (Table 1). Most of the cell lines were checked for mycoplasma contamination at the time of the test: no correlation was found between the presence of mycoplasma and the induction of antiviral activity.

Mouse cell lines were also tested for their ability to induce interferon in human lymphocytes (data not shown). Several were positive, including some continuous cell lines and 5 out of 19 SV40-transformed cell lines. The interferon produced by cultivation of mouse cell lines and human lymphocytes was of human origin, as determined by its ability to inhibit viral replication in human cells and not in mouse cells: this result indicates that the lymphocytes in these mixed cultures and not tumour cells are responsible for interferon production.

The kinetics of interferon production in these experiments were similar to those observed after virus infection of lymphocytes (Fig. 1). Antiviral activity was detectable in the supernatant 4–5 h after the lymphocytes were added to the cell cultures and its titre increased up to 16–24 h. The final titres of antiviral activity varied according to the cell line and the lymphocyte preparation; however, the positive cell lines induced interferon production by each lymphocyte preparation and the negative cell lines never induced interferon.

The virus inhibitor in the supernatants of the mixed cultures meets all the criteria established by Lockart<sup>24</sup> to be considered as an interferon: it is species-specific, destroyed by trypsin, resistant to treatment at pH 2 and the active components can be eluted from a Sephadex G-100 column in two fractions corresponding to





**Fig. 1** Kinetics of interferon production from human lymphocytes infected with Newcastle disease virus or cultured with a human rhabdomyosarcoma-derived cell line. Human peripheral blood lymphocytes were isolated on a Ficoll-Hypaque gradient. Adherent cells were removed by two incubations (1 h each, 37 °C) on glass Petri dishes. The cells were resuspended at  $10^7$  ml $^{-1}$  in RPMI 1640 medium with 10% foetal bovine serum and 1 ml of medium containing cells was added to 16 mm wells (Linbro Disposo trays FB16-24TC) empty or containing a monolayer of human rhabdomyosarcoma-derived cells (cell line RDMC). The supernatants were taken at the indicated times, centrifuged at 1,000g for 30 min and tested for antiviral activity. Supernatants from Newcastle disease virus-infected lymphocytes were treated at pH 2 for 4 d and then neutralised before testing. The antiviral activity was expressed in terms of the cytopathic effect, 50% end point inhibition test. Serial dilutions (100  $\mu$ l) of the supernatant were added to the wells of microtitre plates (Microtitre II, Falcon) containing monolayers of human foetal skin fibroblasts. After overnight incubation the supernatants were removed and 50,000 plaque-forming units of vesicular stomatitis virus (Indiana strain) were added in a volume of 0.2 ml. The final evaluation of cytopathic effect was done after 48 h. Each assay included a standard interferon preparation (NIH human reference interferon G-023-901-527 with a titre of 20,000 units). Supernatants from: ●, lymphocytes alone; □, RDMC monolayer alone; ■, mixed culture lymphocytes and RDMC; ○, mixed culture lymphocytes and RDMC in presence of cycloheximide 100  $\mu$ g ml $^{-1}$ ; ▲, lymphocytes infected with Newcastle disease virus at multiplicity of infection of 10.

molecular weights of approximately 45,000 and 25,000. Inhibition of cellular protein synthesis, by the addition of cycloheximide to the lymphocyte-tumour cell culture, suppressed interferon production (Fig. 1). In addition to the antiviral activity, the supernatants from the mixed cultures inhibit cell growth and DNA synthesis in human cell lines and have an effect on the cytotoxic activity of human peripheral blood lymphocytes (manuscript in preparation). These activities, which have also been described with purified preparation of virus-induced interferons<sup>13-16,25</sup>, in the supernatants from lymphocyte-cell line mixed cultures were positively correlated with the antiviral activity and were eluted in the same two peaks after gel filtration.

The stimulus for production of interferon by lymphocytes on incubation with cell lines is unknown. Contact between lymphocytes and cells seems to be required because supernatants from the cell lines are unable to induce interferon production by lymphocytes. When human cells were used as inducers, with the exceptions of two SV40-transformed cell lines, only tumour-derived lines were able to induce antiviral activity in human lymphocytes.

For two types of human tumours, melanoma and colorectal

adenocarcinoma, various cell lines from different patients were analysed. All four melanoma-derived cell lines tested induced interferon. Of the six colorectal carcinoma-derived cell lines, only two were positive. These two positive cell lines were derived from the same patient<sup>20</sup>—line SW480 from the primary adenocarcinoma of the colon, and line SW620 from metastases in the lymph node of the same patient. The other four colorectal carcinoma cell lines tested did not induce interferon.

The allogeneic (or xenogeneic) antigens on the surface of the cells were probably not responsible for interferon stimulation. Preliminary experiments with autologous cells in humans and with syngeneic cells in a murine system have shown that interferon is produced even in the absence of allogeneic differences between the lymphocytes and the inducer cell line. Moreover, alloantigens are present on both inducing and non-inducing cell lines and in the presence of a strong allogeneic stimulus, as in mixed leukocyte culture, no antiviral activity was detected in the supernatant after 24 h of incubation (Table 1). The induction of interferon by an allogeneic stimulus in mouse mixed leukocyte culture has been reported to occur only after 4–5 d of stimulation and at a titre much lower than that observed in our experiments<sup>9</sup>. 'Tumour'

**Table 1** Induction of interferon by various human cell lines co-cultured with human lymphocytes

Cell line	Ref.	Origin	Passages	Lymphocyte preparations tested	Units interferon induced*
6 preparations		Peripheral blood lymphocytes†		6	<1
2 strains		Foetal skin fibroblasts	6–40	6–12	<1
3 strains		Foetal lung fibroblasts	20–40	4–8	<1
2 strains		Brain	2–15	4	<1
7 strains		Skin fibroblasts	5–40	4	<1
4 lines	17,18	SV40-transformed fibroblasts	20–240	4–8	<1
9 lines	19	Brain, SV40-transformed	4–22	4	<1
S1054TR	19	Brain, SV40-transformed	32	4	<1
S1003 3WTR	19	Brain, SV40-transformed	8	4	193 ± 137
4 lines	20	Colorectal carcinoma	10–95	4	125 ± 0
SW620	20	Colorectal carcinoma	160–165	4	<1
SW480	20	Colorectal carcinoma	105–110	3	362 ± 137
SW690		Melanoma	80–90	5	125 ± 0
SW691		Melanoma	80–85	4	850 ± 552
SW843		Melanoma	30–40	4	6,000 ± 3,000
SW489		Melanoma	30–40	3	244 ± 119
RDMC	21	Rhabdomyosarcoma	150–200	32	25 ± 0
HT1080	22	Fibrosarcoma	110–115	4	3,494 ± 1,008
D98 (HeLa)	23	Cervical carcinoma	50–60	4	<1
					312 ± 165

Monolayers of the different cell lines have been co-cultured with various human lymphocyte preparations as indicated in Fig. 1.

\*Antiviral activity was measured in the supernatants after 24 h of co-cultivation; values are means ± s.e.m.

†Equal volumes of allogeneic lymphocyte preparations from peripheral blood ( $10^7$  cells ml $^{-1}$ ) were mixed and the supernatants collected after 24 h as in the lymphocyte—cell line mixed cultures.

antigens present on the surface of the inducer cells might be responsible for an immune stimulation of interferon. But, the ability of the lymphocyte preparations from all donors tested to produce interferon when cultured with inducer cell lines, the high titre of interferon produced and the early kinetics of production seem to differentiate the mechanism of interferon induction described in this study from other known systems of immune stimulation; for example, stimulation of primed human lymphocytes with various viral antigens induces only low levels of interferon (less than 100 units in most systems) after 5–7 d of stimulation<sup>6,7</sup> and then only in lymphocytes from immune donors. Particular cell-surface characteristics in the positive cell lines might be responsible for the interaction with the lymphocytes and for their stimulation. The presence in the lymphocyte population of natural killer cells that are known to interact with and lyse some of the tumour cells during incubation<sup>2,6</sup> might be responsible for induction or release of the factor(s) responsible for interferon induction. The possibility that viruses present on the human cell lines are the inducers, although unlikely, cannot be excluded.

The ability of interferon to enhance specific and nonspecific cell-mediated cytotoxicity, as well as its inhibitory effect on tumour cell growth could be responsible for the antitumour effect *in vivo* observed with exogenous interferon and with interferon inducers. If, as observed with tumour-derived cell lines *in vitro*, tumour cells *in vivo* are able to interact with the host lymphocytes and induce interferon production, it is possible that this induction of endogenous interferon is one of the natural defense mechanisms of the host against tumour growth and invasion.

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- Youngner, J. S. in *Interferon* (Little Brown, Boston, 1970).
- Merigan, T. C. & Finkelstein, M. S. *Virology* **35**, 363–376 (1968).
- Wheelock, E. F. *Science* **149**, 310–311 (1965).
- Falcoff, R., Orzal, R. & Iscaki, S. *Eur. J. Immun.* **2**, 476–478 (1976).
- Glasgow, L. A. *J. Bact.* **91**, 2185–2191 (1966).
- Rasmussen, L. E., Jordan, G. W., Stevens, D. A. & Merigan, T. C. *J. Immun.* **112**, 728–736 (1974).
- Epstein, L. B., Stevens, D. A. & Merigan, T. C. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2632–2636 (1972).
- Green, J. A., Cooperband, S. R. & Kibrick, S. *Science* **164**, 1415–1417 (1969).
- Gifford, G. E., Tibor, A. & Peavy, D. L. *Infect. Immun.* **3**, 164–166 (1971).
- Atanasiu, P. & Chany, C. C. *r. hébd. Séanc. Acad. Sci., Paris* **251**, 1687–1689 (1960).
- Gresser, I. *et al. C. r. hébd. Séanc. Acad. Sci.* **268**, 994–997 (1969); *Proc. natn. Acad. Sci. U.S.A.* **63**, 51–57 (1969).
- Gresser, I. in *Advances in Cancer Research* (eds Klein, G. & Weinhouse, S.) (Academic, New York and London, 1972).
- Paucker, K., Cantell, K. & Henle, W. *Virology* **17**, 324–334 (1962).
- Chernyakhovskaya, I. Y., Slavina, E. G. & Svet-Molavsky, G. L. *Nature* **228**, 71–72 (1970).
- Lindhal, P., Leary, P. & Gresser, I. *Proc. natn. Acad. Sci. U.S.A.* **69**, 721–725 (1972).
- Huang, K. Y., Donahoe, R. M., Gordon, F. B. & Dressler, H. R. *Infect. Immun.* **4**, 581–588 (1971).
- Weiss, M. C., Ephrussi, B., Scalletta, L. J. *Proc. natn. Acad. Sci. U.S.A.* **59**, 1132–1135 (1968).
- Croce, C. M., Girardi, A. J. & Koprowski, H. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1397–1400 (1975).
- Santoli, D., Wroblewska, Z., Gilden, D. H., Girardi, A. & Koprowski, H. *J. comp. Neurol.* **161**, 317–328 (1975).
- Leibovitz, A. *et al. Cancer Res.* **36**, 4562–4569 (1976).
- McAllister, R. M., Nelson-Rees, W. A., Johnson, E. Y., Rongey, R. W. & Gardner, M. B. *J. natn. Cancer Inst.* **47**, 603–612 (1971).
- Rasheed, S., Nelson-Rees, W. A., Toth, E. M., Arnstein, P. & Gardner, M. B. *Cancer* **33**, 1027–1033 (1974).
- Nelson-Rees, W. A. & Flandermayer, R. R. *Science* **191**, 96–98 (1976).
- Lockart, R. Z., Jr. *Interferons and Interferon Inducers* (ed. N. B. Finter) 11–27 (North-Holland, Amsterdam, 1973).
- Stewart, W. E. II *et al. Nature* **246**, 141–143 (1973).
- Santoli, D., Trinchieri, G., Zmijewski, C. M. & Koprowski, H. *J. Immun.* **117**, 765–770 (1976).

## Activation of human complement by glutaraldehyde-treated red cells

THERE is now much evidence that the classical complement pathway can be activated by substances other than immune complexes. Leonard and Thorne<sup>1</sup> found that precipitate formed between the polyanion, polyglutamic acid, and lysozyme was anticomplementary, although the site of action was not determined. Since then, many polyionic substances and complexes have been found to activate the classical complement pathway, namely polyinosinic acid<sup>2</sup>, dextran sulphate<sup>2,3</sup>, cellulose sulphate<sup>4</sup>, heparin–protamine complexes<sup>5</sup>, lipid A<sup>6</sup>, lysozyme–DNA complexes<sup>7</sup>, double-stranded DNA<sup>8</sup> and C-reactive protein–polycation complexes<sup>9</sup>. Although activation by heparin–protamine complexes was found to take place in agammaglobulinaemic serum<sup>10</sup>, it is possible that the polyions do not react directly with C1, but that they bring about aggregation of IgG, which then combines with and activates C1. On the other hand, C-reactive protein attached to the surface of red cells can activate complement without involvement of antibody or IgG (refs 11, 12), as can certain oncoviruses<sup>13</sup> and mitochondrial membranes<sup>14</sup>. It has also been shown that the complement component C1q will combine with glutaraldehyde-treated red cells and that the binding sites involved on the C1q molecule are probably those also involved in binding to immune complexes<sup>15</sup>. It has now been found that glutaraldehyde-treated red cells not only bind to C1q, but can also activate the complement system through the classical pathway, with no evidence of involvement of immunoglobulin.

Group O Rh-negative red cells were washed six times in saline and treated with glutaraldehyde followed by lysine, as previously described<sup>15</sup>. These cells were added to an equal volume of fresh serum and incubated at 37 °C for 2–24 h. Control samples contained 10 mM EDTA. Aliquots of the supernatant serum were then taken for assessment of complement activity. After incubation for 2 h, there was a reduction in complement haemolytic activity<sup>16</sup> (CH<sub>50</sub>) to 25–50% compared with that of the controls. Analysis of the complement components C2 (using a human homozygous C2-deficient serum), C4 (using ammonia-treated guinea pig serum<sup>16</sup>) and C3 and other late-acting components (using zymosan-treated guinea pig serum<sup>16</sup>) showed a progressive reduction in all components with almost complete loss of activity at 16–24 h. C3 breakdown products were also shown to be present in the serum after incubation for 6 h with glutaraldehyde-treated red cells, using the crossed immunoelectrophoretic method<sup>18</sup>, with an antiserum containing anti-C3b, -C3c and -C3d. As Fig. 1 shows, there was almost complete conversion of C3 to C3c. Small

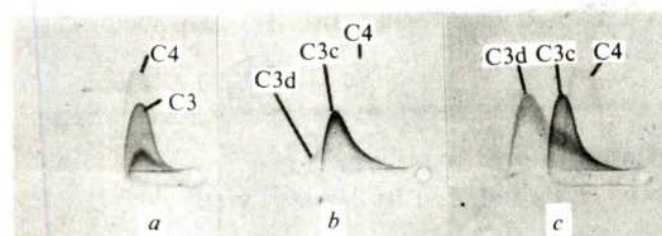


Fig. 1 Crossed immunoelectrophoresis of fresh serum after incubation for 6 h with glutaraldehyde-treated red cells, a, in the presence of 10 mM EDTA, and b, without EDTA. c, A serum aged for 4 d at 37 °C, used for identification of C3c and C3d precipitation lines. The antiserum contained antibodies specific for C3, C3c, C3d and C4.



amounts of C3d were present in the serum, but most was attached to the red cells, as shown by using an  $^{125}\text{I}$ -labelled anti-C3d (ref. 17). The C1q content of the serum was reduced to barely detectable levels, as shown by single radial diffusion assay using anti-C1q.

These results are typical of those found with activation of complement through the classical pathway. It is improbable that the complement system was activated through IgG fixed to the red cells, as the cells were thoroughly washed before glutaraldehyde treatment, and only trace amounts of IgG could have been present on their surface. Activation of complement through IgG on the surface of membrane requires substantial amounts of antibody to be present; for instance, about 5,000 molecules of IgG anti-A are required on each red cell before complement components can be demonstrated to be present on the red cell surface<sup>19</sup>. Thus, it is unlikely that complement is being activated through IgG bound to the cells and it is more probable that the site for binding and activation of C1 is an integral part of the red cell membrane.

The ability of complement to be activated *in vitro* through the classical pathway by glutaraldehyde-treated red cells, as well as by oncovirus and mitochondria without the intervention of IgG, indicates that complement has the potential to act *in vivo* independently of other systems in the elimination of foreign membranous particles. Verhoef *et al.*<sup>20</sup> have provided evidence that the classical complement pathway can be activated by direct reaction with bacterial cell wall protein A, resulting in opsonisation and phagocytosis of the organisms.

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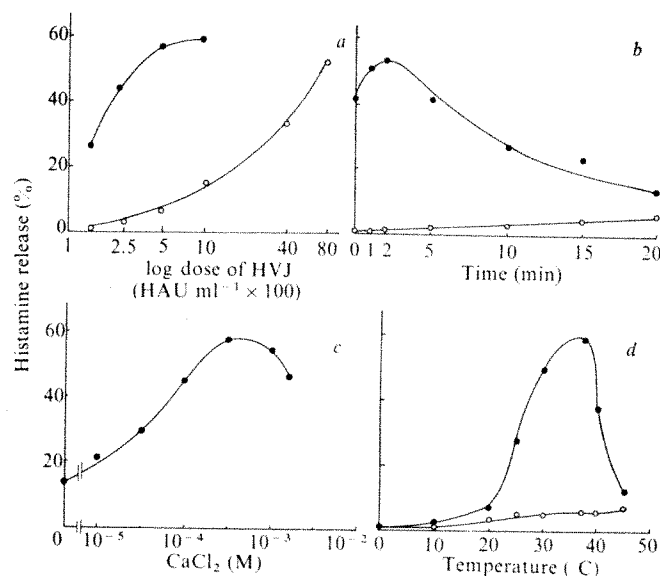
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- Leonard, C. G. & Thorne, C. B. *J. Immun.* **87**, 175-188 (1961).
- Yachnin, S. & Rutenberg, J. M. *J. clin. Invest.* **44**, 518-534 (1965).
- Loos, M. & Bitter-Seurmann, D. *Immunology* **31**, 931-934 (1976).
- Eisen, V. & Loveday, C. *Br. J. Pharmac.* **39**, 831-833 (1970).
- Rent, R., Ertel, N., Eisenstein, R. & Gewurz, H. *J. Immun.* **114**, 120-124 (1975).
- Lachman, P. J. & Nicol, P. in *Advances in Biosciences* **12**, (ed. G. Raspe) (Pergamon, Oxford, 1974).
- Willoughby, W. F., Ford, R. T. & Shin, H. S. *J. Immun.* **111**, 296-297 (1973).
- Cooper, N. R. in *Contemporary Topics in Molecular Immunology* **2** (ed. R. A. Reisfeld & W. J. Mandy) (Plenum, New York, London, 1973).
- Siegel, J., Osmand, A. P., Wilson, M. F. & Gewurz, H. *J. exp. Med.* **142**, 709-719 (1975).
- Fiedel, B. A., Rent, R., Myhrman, R. & Gewurz, H. *Immunology* **30**, 161-169 (1976).
- Kaplan, M. H. & Volanakis, J. E. *J. Immun.* **112**, 2135-2147 (1974).
- Osmand, A. P., Mortenson, R. F., Siegel, J. & Gewurz, H. *J. exp. Med.* **142**, 1065-1076 (1975).
- Cooper, N. R., Jensen, F. C., Welsh, R. M. & Oldstone, M. B. A. *J. exp. Med.* **144**, 970-984 (1976).
- Pinckard, R. N. *et al. J. Immun.* **110**, 1376-1382 (1973).
- Hughes-Jones, N. C. *Immunology* **32**, 191-198 (1977).
- Lachman, P. J., Hobart, M. J. & Aston, W. P. in *Handbook of Experimental Immunology* (ed. D. M. Weir) (Blackwell, Oxford 1973).
- Chaplin, H., Freedman, J. & Hughes-Jones, N. C. *Immunology* **32**, 1007-1015 (1977).
- Laurell, C. B. *Analyt. Biochem.* **10**, 358-361 (1965).
- Romano, E. L. & Mollison, P. L. *Br. J. Haemat.* **29**, 121-127 (1975).
- Verhoef, J., Peterson, P. K., Kim, Y., Sabath, L. D. & Quie, P. G. *Immunology* **33**, 191-197 (1977).



**Fig. 1** *a*, Histamine release by HVJ and its potentiation by phosphatidylserine (PS). Isolated rat mast cells ( $1 \times 10^5$ – $2 \times 10^5$  cells) were incubated for 10 min at 37 °C in varying concentrations of HVJ in PBS, with or without PS (10  $\mu\text{g}$ ). Total reaction volume was 1 ml. ○, Histamine release by HVJ; ●, histamine release by HVJ and PS. Values for percentage histamine release are the means of experiments corrected for spontaneous release. *b*, Effect of PS on histamine release by HVJ in relation to the time of addition of PS. Cells were incubated for varying times at 37 °C with HVJ (500 HAU ml<sup>-1</sup>) in PBS, then PS (10  $\mu\text{g}$  ml<sup>-1</sup>) was added for a further incubation period of 10 min. ●, Histamine release by HVJ and PS; ○, spontaneous release. *c*, Effect of  $\text{Ca}^{2+}$  on histamine release by HVJ and PS. Cells were incubated for 2 min at 37 °C with HVJ (500 HAU ml<sup>-1</sup>) in PBS containing  $\text{CaCl}_2$  in varying concentrations, then PS (10  $\mu\text{g}$  ml<sup>-1</sup>) was added for a further incubation period of 10 min. *d*, Effect of temperature on histamine release by HVJ and PS. Cells were incubated for 2 min at varying temperatures with HVJ (500 HAU ml<sup>-1</sup>) then PS (10  $\mu\text{g}$  ml<sup>-1</sup>) was added for a further 10 min incubation. ●, Histamine release by HVJ and PS; ○, spontaneous release.

occurred frequently. This observation led to the theory that histamine release from mast cells was induced by virus, as well as by antigen from sensitised mast cells and by histamine releasers. The results reported here indicate that histamine release from mast cells was induced by direct exposure of cells to virus in the presence of  $\text{Ca}^{2+}$  at 37 °C and that it was markedly enhanced by the addition of phosphatidylserine (PS), which is a selective enhancer<sup>3</sup> of anaphylactic histamine release.

Mast cells were isolated from rat peritoneal fluid by the method as described before<sup>4</sup> and suspended in a phosphate-buffered saline (PBS, 154 mM NaCl, 2.7 mM KCl, 0.9 mM  $\text{CaCl}_2$ , 6.7 mM  $\text{KH}_2\text{PO}_4$ – $\text{Na}_2\text{HPO}_4$ , pH 7.2 and 0.01% bovine serum albumin). HVJ-z strain was propagated in embryonated eggs and concentrated by a centrifugation<sup>5</sup>. The mast cell suspension was incubated with HVJ for 2 min at 37 °C and then PS, sonicated in PBS, was added. After 10 min of incubation, the reaction was terminated by adding 2 ml of ice-cold PBS and the reaction mixture was centrifuged at 3,000g for 20 min. Histamine in the supernatants and precipitates was determined according to the fluorescence method of Shore *et al.*<sup>6</sup>. Lactate dehydrogenase (LDH) activity was determined by the method of Burch *et al.*<sup>7</sup>.

Incubation of HVJ suspension (final virus concentration, 8,000 HAU ml<sup>-1</sup>) with mast cells for 10 min at 37 °C induced histamine release accompanied by degranulation from the cells. With a concentration of 500 HAU ml<sup>-1</sup> of HVJ, histamine release hardly occurred, but the addition of 10  $\mu\text{g}$  ml<sup>-1</sup> PS to such a cell suspension resulted in a marked histamine release (Fig. 1*a*), this release being almost com-

## Histamine release from rat mast cells induced by Sendai virus

SENDAI virus (HVJ, haemagglutinating virus of Japan), a type 1 parainfluenza myxovirus, agglutinates certain types of cells and induces cell fusion<sup>1</sup>. Previously<sup>2</sup> we reported on the degranulating activity in the hybrid cells derived from rat mast cells and Ehrlich ascites tumour cells using HVJ. During that work, it was found that histamine release accompanied by degranulation from unfused mast cells



plete within 5 min. The histamine release induced by HVJ and PS was dependent on the time at which PS was added. Figure 1b shows that when PS was added after a 2-min exposure of mast cells to virus, histamine release reached a maximum and then decreased on further exposure to virus. Phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acid were all ineffective in this respect.

Calcium ions were necessary for both the histamine release by HVJ and the potentiation by PS of HVJ-induced histamine release from mast cells. As shown in Fig. 1c, the effect of calcium increased with the increase of concentration from  $10^{-3}$  M, reaching maximum at around  $5 \times 10^{-4}$  M. Histamine release induced by HVJ and PS in the presence of  $\text{Ca}^{2+}$  was dependent on temperature (Fig. 1d), the optimum being  $37^\circ\text{C}$ . At temperatures below  $20^\circ\text{C}$  and above  $45^\circ\text{C}$ , histamine release was inhibited.

The specificity of HVJ in histamine-releasing activity was examined. Adsorption of the virus to human erythrocytes resulted in complete inhibition of histamine-releasing activity of the virus. This activity was not, however, affected by repeated washing of the virus by centrifugation and resuspension. Histamine release from the mast cells induced by  $500 \text{ HAU ml}^{-1}$  HVJ was not accompanied by the release of LDH, but a prolonged incubation with a high concentration of HVJ ( $8,000 \text{ HAU ml}^{-1}$ ), did result in the release of LDH.

These observations indicate that histamine release from mast cells is directly induced by virus and that this release is similar to antigen-, dextran- and concanavalin A-induced reactions<sup>3,8,9</sup>. These reactions are dependent on  $\text{Ca}^{2+}$  and temperature, and are potentiated by PS. It is therefore suggested that a common process, which is related to membrane function leading to exocytosis by fusion of the plasma and granule membrane, is involved in the mechanism of their histamine release.

It is well known that HVJ possesses haemolytic activity, but histamine release from mast cells by this virus could not be caused by its lytic action. This point is clear from the fact that HVJ-induced histamine release from mast cells was not accompanied by the leakage of LDH. Moreover,  $\text{Ca}^{2+}$  required in this histamine release acts in an inhibitory manner on the haemolytic activity of HVJ, and such haemolysis requires phosphatidylethanolamine; PS is not effective<sup>10</sup>. Recently, Homma *et al.*<sup>11</sup> have prepared HVJ that possesses cell fusion full activity and infectivity, but no haemolytic activity. A comparative study of the histamine release from mast cells is now being carried out using non-haemolytic and haemolytic preparations.

The mechanism of histamine release by HVJ and enhancement by PS, in the presence of  $\text{Ca}^{2+}$  still remains unclarified. The study, by immuno-freeze-etching, of the fusion of erythrocytes by HVJ has elucidated that when viral envelopes fuse with red blood cell membranes, agglutination of intermembranous glycoproteins occurs<sup>12</sup>. Chi *et al.*<sup>13</sup> have demonstrated that a displacement of intramembranous particles occurs at an early stage in the secretory reaction of mast cells. From these findings it seems that even at an early stage of infection of mast cells by HVJ there probably occurs a perturbation of the membrane structure, followed by clustering of intermembranous particles. It has been suggested that fusion of granule membrane and cell membrane takes place at the region of perturbed lipid bilayer denuded of intermembranous particles<sup>14</sup>.  $\text{Ca}^{2+}$  and PS in all probability, play a part in the enhancement of the formation of fluid cluster in the membrane, or the perturbation of lipid bilayer<sup>15</sup>. If PS is absent, the perturbation in the membrane induced by HVJ would rapidly be stabilised (Fig. 1b). The fact that histamine release induced by HVJ is highly sensitive to temperature also seems to indicate that membrane fluidity is important in membrane fusion.

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- Okada, Y., Suzuki, T. & Hosaka, Y. *J. Med. Osaka Univ.* 7, 709-717 (1957).
- Sugiyama, K. *Experientia* 32, 1402-1403 (1976).
- Goth, A., Adams, H. R. & Knoohuizen, M. *Science* 173, 1034-1035 (1971).
- Sugiyama, K. *Jap. J. Pharmac.* 21, 209-226 (1971).
- Okada, Y. *Exp. Cell Res.* 26, 98-107 (1967).
- Shore, P. A., Burkhalter, A. & Cohn, V. H. *J. Pharmac. exp. Ther.* 127, 182-186 (1959).
- Burch, H. B. *et al. J. biol. Chem.* 238, 2267-2273 (1963).
- Mongar, J. L. & Svec, P. *Br. J. Pharmac.* 47, 741-752 (1972).
- Sugiyama, K., Sasaki, J. & Yamasaki, H. *Jap. J. Pharmac.* 25, 485-487 (1975).
- Hosaka, Y. & Shimizu, Y. K. *Virology* 49, 640-646 (1972).
- Homma, M., Shimizu, K., Shimizu, Y. K. & Ishida, N. *Virology* 71, 41-47 (1976).
- Bachi, T., Aguet, M. & Howe, C. J. *Viol.* 11, 1004-1012 (1973).
- Chi, E. Y., Lagunoff, D. & Koehler, J. K. *Proc. natn. Acad. Sci. U.S.A.* 73, 2823-2827 (1976).
- Akhong, Q. F., Fisher, D., Tampion, W. & Lucy, J. A. *Nature* 253, 194-195 (1975).
- Ohnishi, S. I. & Ito, T. *Biochemistry* 13, 881-887 (1974).

## Immediate early antigens in human cytomegalovirus infected cells

DURING a comparison of cytomegalovirus (CMV)-specific haemagglutinating and immunofluorescent antibody titres in human sera, 3 of 60 sera tested by indirect immunofluorescence gave homogenous nuclear staining in 100% of infected cells within 1 h after infection (p.i.). These antigens differ from EBNA-type antigens described recently by Geder<sup>1</sup> in CMV-infected cells 3 h p.i. in that anticomplement immunofluorescence<sup>2</sup> is not required for their detection. They differ from 'early antigens' (EA)<sup>3</sup> in that they appear much sooner and are located uniquely in the nucleus. Finally, they differ from 'late antigens' (LA) for they appear in the absence of DNA synthesis. By analogy to antigens described for the pseudo-rabies system by Ben-Porat *et al.*<sup>4</sup> and for herpes simplex virus type 1 by Beth *et al.*<sup>5</sup>, we term these antigens 'immediate early antigens' (IEA).

CMV-IEA were partially characterised by studying their appearance in cells treated with inhibitors of DNA, RNA and protein synthesis, as well as in cells non-permissive for human CMV replication. The titres of each serum in antibodies to IEA, EA and LA is given in Table 1. The reactivity of two of the three IEA-positive sera (M19 and ICM) was studied in various conditions and compared with that of an IEA-negative serum (AUD).

When human diploid cells (MRC-5) were infected with 1 PFU (plaque-forming unit) per cell of Mira strain CMV, diffuse nuclear fluorescence was detected 1 h later using a 1/60 dilution of M19 or ICM (Fig. 1a). The serum AUD (diluted 1/60, Fig. 1b) failed to detect any intracellular antigens until 15 h after infection. Appearance of antigens detectable by IEA-positive sera was not influenced by treatment of cells before or after infection with inhibitors of DNA or RNA synthesis. Neither cytosine arabinoside ( $20 \mu\text{g ml}^{-1}$ ) nor iododeoxyuridine ( $100 \mu\text{g ml}^{-1}$ ) added at the time of infection, nor phosphonoacetic acid ( $50 \mu\text{g ml}^{-1}$ ) added 24 h before and maintained after infection affected IEA formation. Actinomycin D added at the time of infection at  $6-20 \mu\text{g ml}^{-1}$  failed to inhibit antigen formation even when cells were pretreated with this drug ( $10 \mu\text{g ml}^{-1}$ ) for as long as 15 h before infection. Cycloheximide when added 18 h before infection and maintained after infection blocked antigen formation; however, when cycloheximide was added only 1 h before infection and maintained in the culture medium after infection, antigen formation was not blocked.



IEA-positive sera detected antigens induced by two strains of CMV (Mira and K9V), but not by HSV-1 (KOS and A 44 strains), HSV-2, or by a bat herpesvirus (Eidolon)<sup>7</sup> when tested 3 h after infection at a dose of approximately 1 PFU per cell. Neutralisation of Mira strain CMV with either MIC or ICM at 37 °C for 1 h eliminated nuclear fluorescence. Incubation of virus at 37 °C for 1 h in growth medium or with a CMV-negative serum did not affect appearance of IEA fluorescence.

Ultraviolet light irradiation (30 min at 85 erg mm<sup>-2</sup> s<sup>-1</sup>) and heat inactivation (30 min at 56 °C) of infecting virus

**Table 1** Immunofluorescence titres of sera used to study immediate early (IEA) and late (LA) antigens in human cytomegalovirus infected cells

Serum	IEA*	CMF specific antigens	
		LA†	IgM†
M19	320	320	80
MIC	20	80	ND
ICM	80	320	0
AUD	0	320	ND

Human diploid cells (MRC-5) were grown in Leibovitz L-15 medium supplemented with 10% calf serum and infected with 1 PFU per cell of human cytomegalovirus by plating on to coverslips 0.2 ml of virus and 2 × 10<sup>6</sup> cells in 0.2 ml in each well of 25-well Sterilin culture plates. Immunofluorescence was carried out 3 h p.i. when seeking IEA and 72 h p.i. when estimating LA and CMV-specific IgM antibody activity. All cells were washed in three changes of phosphate-buffered saline (PBS) containing Mg<sup>3+</sup> and Ca<sup>2+</sup> ions and fixed in two changes of ice cold methanol for a total of 5 min. Coverslips were air-dried, incubated with appropriate dilutions of test sera for 30 min at 37 °C when testing for IEA and LA, and for 3 h at 37 °C when testing for IgM. Coverslips were then washed in at least four changes of PBS over a period of 10 min and incubated with either FITC-conjugated goat-anti-human IgG (Melory) or with FITC-conjugated goat-anti-human IgM (Melory) for 30 min at 37 °C. After extensive washing in PBS, coverslips were fixed in 10% formol, washed with PBS and mounted in glycerine/PBS (9:1) mixture.

\*Titres expressed as reciprocal of the highest dilution giving whole nuclear fluorescence.

†Titres expressed as reciprocal of the highest dilution giving ++ (maximum +++ fluorescence of nuclear inclusion bodies).

prevented IEA formation. Ultraviolet irradiation of cells for 10 or 20 min before infection did not inhibit antigen appearance. Nuclear fluorescence varied as a function of virus dilution; a 1/5 dilution gave 70% fluorescence and a 1/10 gave 20% fluorescence.

Human CMV induced IEA in non-permissive cells. Mouse fibroblasts (MLF-CBA, isolated at the Pasteur Institute) and epithelial cells (TCC<sub>36</sub>, Pasteur Institute) reacted positively with IEA-positive sera 18 and 24 h, respectively, after infection with Mira strain CMV. IEA-negative serum revealed no antigens in these cells at any of the times studied. Rabbit ear skin fibroblasts (RSF, Pasteur Institute) showed 100% nuclear fluorescence 9 h after infection with either Mira or K9V CMV (Fig. 1c, d). In these cells, IEA-positive fluorescence disappeared on ultraviolet irradiation of infecting virus. None of the sera used in this study reacted positively with CMV transformed hamster fibroblasts (Cx90 3B) at passage 39.

If iododeoxyuridine (IUdR) 100 µg ml<sup>-1</sup> applied at the time of infection was used to block DNA synthesis, IEA disappeared from nuclei and appeared in the cytoplasm 45 h after infection, at which site they were barely detectable 72 h p.i. This would suggest that in the absence of DNA synthesis, IEA either disappear or lose their original antigenic structure after leaving the nucleus. IEA are probably not structural proteins of the virus since they are practically undetectable in cells blocked by IUdR 72 h after infection at a time when IEA-negative LA-positive serum detects antigens in both nucleus and cytoplasm. IEA might be related to CMV-induced stimulation of cellular protein synthesis known to occur early after infection<sup>8</sup>.

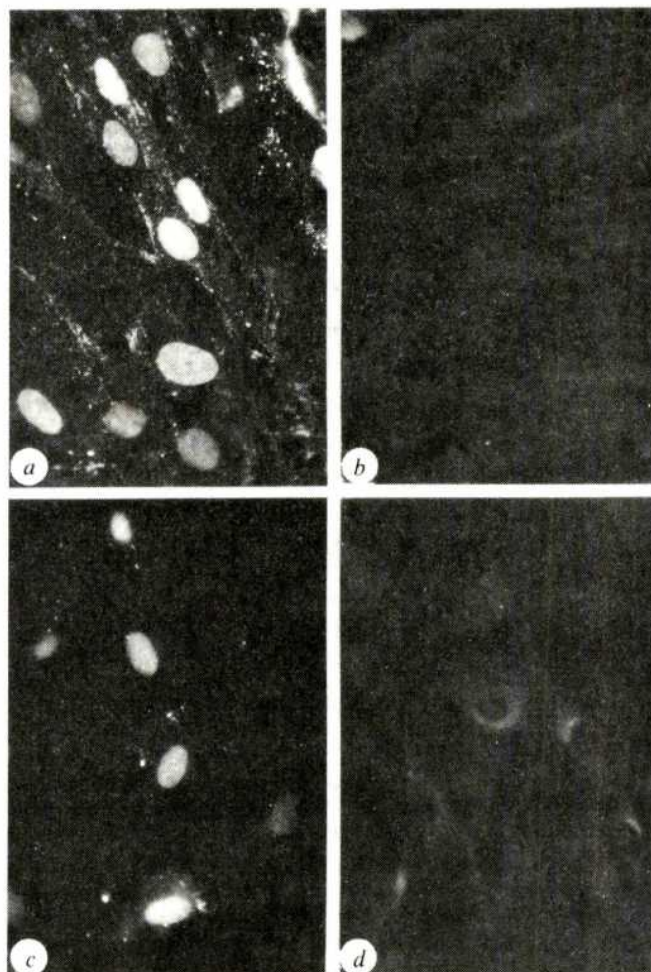
The significance of these antigens in the CMV replicative cycle remains obscure for three reasons. First, the abundance of these antigens in the nucleus within such a short time p.i. when viral DNA synthesis has not occurred. Second, these antigens appear in spite of inhibition of DNA and DNA-dependent RNA synthesis. And third, ultraviolet inactivation experiments show that functional virus DNA is required. The possibility that these antigens represent accumulation of infecting viral capsids in the nucleus seems to be ruled out since IEA-negative LA-positive serum gives no nuclear fluorescence and as electron microscope examination of cells 3 h after infection failed to show any viral capsids in cell nuclei.

IEA antibodies are rarely detectable in CMV positive sera. Among the donors of the three IEA-positive sera, there seems to be no common clinical background which would permit extrapolating from the patient's histories a coherent explanation for the relationship of these antibodies to the stage of disease.

Further characterisation of IEA must await polyacrylamide gel analysis and the production of specific antisera in order to characterise immunoprecipitates. This work is in progress.

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**Fig. 1** Immunofluorescence of HCMV infected cells. *a*, Human lung fibroblasts (MRC-5)-IEA positive serum (M19 1/60) 3 h after infection. *b*, Same cells IEA-negative serum (AUD 1/60). *c*, Rabbit skin fibroblasts 18 h p.i.-IEA-positive serum (M19 1/60). *d*, Same rabbits cells-IEA-negative serum (AUD 1/60). (× 1,000).



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1. Geder, L. *J. gen. Virol.* 32, 315-319 (1976).
2. Reedman, B. M. & Klein, G. *Int. J. Cancer* 2, 488-520 (1973).
3. The, T. H., Klein, G. & Langenhuyzen, M. A. C. *Clin. exp. Immun.* 16, 1-12 (1974).
4. Ben-Porat, T., Kervina, M. & Kaplan, A. S. *Virology* 65, 355-362 (1975).
5. Beth, E., Giraldo, G. & Bernard, M. *Biomedicine* 25, 366-368 (1976).
6. Giraldo, G., Beth, E. & Haguenu, F. *J. natn. Cancer Inst.* 49, 1506-1526 (1972).
7. Guillon, J. C., Vincent, J. & Boche, R. *Bull. Inst. Pasteur* 73, 45-63 (1975).
8. Furukawa, T., Fioretti, A. & Plotkin, S. A. *J. Virol.* 11, 991-99 (1973).

## A single gene determines the host range of influenza virus

THE genome of influenza virus consists of eight distinct segments of single stranded RNA as determined by electrophoresis in polyacrylamide gels<sup>1-3</sup>. Each of these segments codes for one of the eight virus specified polypeptides<sup>4-9</sup> and is therefore believed to be a single gene which functions by a monocistronic messenger RNA<sup>10</sup>. Because different strains of influenza may vary in base sequence homology<sup>9</sup> or in the electrophoretic mobilities of their RNA segments and infected cell polypeptides<sup>11,12</sup>, it is often possible to determine the parental origin of every gene in an interstrain recombinant. Such procedures have been used to investigate the physiological properties associated with some of the influenza virus specified proteins<sup>9,13,14</sup>. I describe here the application of this approach to an investigation into the genetic control of host range. The results show that the difference in host range between two avian influenza viruses depends solely on the properties of one of the internal viral proteins associated with RNA polymerase activity.

One of the major areas of interest in the study of influenza viruses is the genetic control of biological properties such as virulence<sup>15</sup>, pathogenicity<sup>16</sup>, plaque morphology<sup>17</sup> and host range<sup>18</sup>. The gene coding for the major surface glycoprotein, the haemagglutinin, may be important in some of these properties. Proteolytic cleavage of the haemagglutinin is necessary for activation of progeny virions<sup>19,20</sup> and is therefore required for multicycle growth in tissue culture and perhaps in the host animal. Since the degree of cleavage of the haemagglutinin varies between different influenza strains and in different cell types<sup>21,22</sup>, Klenk<sup>19</sup> has suggested that cell proteases may play an important part in the host range and spread of infection of these viruses. The pathogenicity of influenza viruses however, seems to be influenced by additional factors. Rott *et al.*<sup>16</sup> have shown that the pathogenicity of fowl plague virus (an avian influenza A virus) is not exclusively dependent on the haemagglutinin nor on the other surface glycoprotein, the neuraminidase, and have suggested that an optimal combination of genes is necessary. Of considerable importance in determining whether a virus has the capacity to become a virulent pathogen would be a gene controlling its host range. Although the above already recognised factors are of undoubted importance, the presence of the correct host range gene would be an essential prerequisite for the emergence of a new strain virulent for a given host. This paper demonstrates that a single gene in influenza can effectively control host range as measured by plaque formation in tissue culture monolayers.

Two avian influenza A viruses (fowl plague virus; FPV) strains Rostock and Dobson were used. Both strains form clear plaques on chick embryo fibroblasts (CEF) but only FPV Dobson forms plaques on the mammalian derived BHK cells. Differences in the ability of influenza strains to form plaques is not uncommon, for example, A/WSN strain forms plaques in MDCK cells and CEF, whereas A/PR/8/34 strain forms plaques in MDCK cells<sup>23</sup> but not CEF<sup>24</sup>. This phenomenon in many cases, however, has previously been attributed to the properties of the viral haemagglutinin and its degree of cleavage in the different cell types. In fact, plaque formation *in vitro* can be induced artificially in some cases by the addition of a proteolytic enzyme, such as trypsin, to the overlay medium<sup>24</sup>.

The ability of FPV Dobson to form plaques in BHK cell monolayers did not depend on the properties of the haemag-

Table 1 Genotypes and plaque assay titres of FPV Rostock and FPV Dobson parents and recombinants

Virus	P <sub>1</sub>	P <sub>2</sub> and P <sub>3</sub>	Parental origin of proteins					Plaque titres		
			HA	NP	NA	M	NS	CEF	BHK	
Rostock	R	R <sup>2</sup>	R <sup>3</sup>	R	R	R	R	R	9.5 × 10 <sup>7</sup>	0
Dobson	D	D <sup>2</sup>	D <sup>3</sup>	D	D	D	D	D	1.4 × 10 <sup>9</sup>	8.0 × 10 <sup>8</sup>
Recombinants										
Di47c-1	R	R <sup>2</sup>	R <sup>3</sup>	R	R	R	R	D	7.5 × 10 <sup>7</sup>	0
DiUS4-5	R	R <sup>2</sup>	R <sup>3</sup>	D	R	R	?	R	1.8 × 10 <sup>8</sup>	0
DiUS1c-2	R	R <sup>2</sup>	R <sup>3</sup>	R	D	R	?	R	1.3 × 10 <sup>8</sup>	0
Di39c-5	D	R <sup>2</sup>	R <sup>3</sup>	R	D	R	?	R	1.9 × 10 <sup>8</sup>	0
DiMn5c-1	D	D <sup>3</sup>	R <sup>3</sup>	R	R	R	?	R	2.6 × 10 <sup>8</sup>	1.4 × 10 <sup>8</sup>
DiMn5c-3	R	D <sup>3</sup>	R <sup>3</sup>	R	D	R	?	R	1.9 × 10 <sup>8</sup>	1.5 × 10 <sup>8</sup>
Di39c-7	?	R <sup>2</sup>	R <sup>3</sup>	R	D	R	?	D	1.2 × 10 <sup>8</sup>	0
Di44c-4	D	D <sup>3</sup>	R <sup>3</sup>	R	D	R	R	R	9.0 × 10 <sup>7</sup>	1.0 × 10 <sup>8</sup>
Dd45-n	R	D <sup>2</sup>	R <sup>2</sup>	D	D	?	?	R	4.3 × 10 <sup>7</sup>	0
Di44-2	R	D <sup>3</sup>	R <sup>3</sup>	R	D	R	?	D	2.9 × 10 <sup>8</sup>	1.6 × 10 <sup>8</sup>
Di44c-1	R	D <sup>3</sup>	R <sup>3</sup>	D	D	R	?	D	3.3 × 10 <sup>7</sup>	5.5 × 10 <sup>7</sup>
DiMn5c-6	D	D <sup>3</sup>	R <sup>3</sup>	D	D	R	?	D	4.5 × 10 <sup>8</sup>	2.3 × 10 <sup>8</sup>
R47i-2	D	D <sup>2</sup>	R <sup>2</sup>	D	D	R	?	D	1.1 × 10 <sup>7</sup>	0
Dd45-3	D	D <sup>2</sup>	R <sup>2</sup>	D	D	?	?	D	6.0 × 10 <sup>7</sup>	0
Di44-7	D	D <sup>3</sup>	R <sup>3</sup>	R	D	D	D	D	2.3 × 10 <sup>7</sup>	1.9 × 10 <sup>7</sup>
Dd45-e	D	D <sup>2</sup>	R <sup>2</sup>	D	D	R	D	D	2.4 × 10 <sup>7</sup>	0

The parental origin of proteins was determined for each recombinant by examining the electrophoretic mobilities of their infected cell polypeptides<sup>11</sup>, and their RNA segments of known coding function<sup>8,11</sup>. P<sub>1,2,3</sub>, Polymerase associated proteins<sup>4</sup>; HA, haemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix protein; NS, non-structural protein 1; 0, no detectable plaques at any dilution. D<sup>2</sup>, Dobson P<sub>2</sub>; D<sup>3</sup>, Dobson P<sub>3</sub>; R<sup>2</sup>, Rostock P<sub>2</sub>; R<sup>3</sup>, Rostock P<sub>3</sub>.



glutinin but on the presence of a gene coding for one of the polymerase associated proteins, P3. The method used involved the construction of recombinants between FPV Dobson and FPV Rostock and the determination of the parental origin of each gene or gene product in these recombinants, as has been described previously<sup>11</sup>. Several such hybrids have been tested for their ability to form plaques in BHK cell and CEF monolayers. The plaque titres obtained, together with the genotype of each recombinant are shown in Table 1. In Table 1 the polymerase-associated proteins P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> are numbered purely on the order of their electrophoretic mobilities in polyacrylamide gels<sup>4,10</sup>, and, because mobilities of proteins vary between different influenza virus strains<sup>11,12</sup>, it is not necessarily valid to assume that the three P proteins will migrate in the same order in all strains. Extensive recombination studies in this laboratory have shown that the P<sub>2</sub> of Rostock and the P<sub>3</sub> of Dobson are functionally equivalent. Therefore any recombinant involving these two proteins will have either Rostock P<sub>2</sub> and Dobson P<sub>3</sub> or Rostock P<sub>3</sub> and Dobson P<sub>2</sub>. In Table 1, therefore, D<sup>3</sup> and R<sup>3</sup> refer to the Dobson and Rostock P<sub>3</sub> proteins respectively, and D<sup>2</sup> and R<sup>2</sup> to the P<sub>2</sub> proteins. All the recombinants that form plaques in BHK cell monolayers have the Dobson P<sub>3</sub> protein (D<sup>3</sup>): this is the only viral gene product common to all the BHK plaque-forming recombinants. Furthermore the haemagglutinin (HA) is not involved in this control since recombinants which possess the Dobson P<sub>3</sub> protein form plaques on BHK cells irrespective of the origin of the HA (for example, DiMn5c-1, and Di44c-1) and recombinants which lack this protein but still

possess the Dobson HA, are unable to form plaques. Also pulse chase experiments of infected cell polypeptides (Fig. 1) suggest that the HA of FPV Rostock is cleaved in BHK cells even though it cannot form plaques.

These results strongly suggest that the gene coding for the P<sub>3</sub> protein of Dobson confers the ability to undergo multicycle growth in BHK cell cultures. Further experiments are necessary to establish the exact function of this protein in the infectious cycle but the results of Palese *et al.*<sup>13</sup> suggest that it may be important in the synthesis of virion RNA. The fact that the equivalent protein, P<sub>2</sub> of FPV Rostock, although synthesised, does not function correctly in these mammalian derived cells, may imply a specific host factor interaction with this protein.

It is possible that mutations in other viral genes will be shown to affect selectively multiplication in particular cell types and that the above observations merely reflect the host range limitation of the FPV Rostock strain. On the other hand, the viral gene product recognised here may be a common host range controlling factor in all influenza viruses. If this is so, the study of its mechanism of action should lead to a better understanding of host-virus interactions which occur in influenza virus multiplication<sup>25,27</sup>, and the gene coding for this protein (gene 1) would be worthy of special consideration in the construction of attenuated recombinant viruses for use as live vaccine in man.

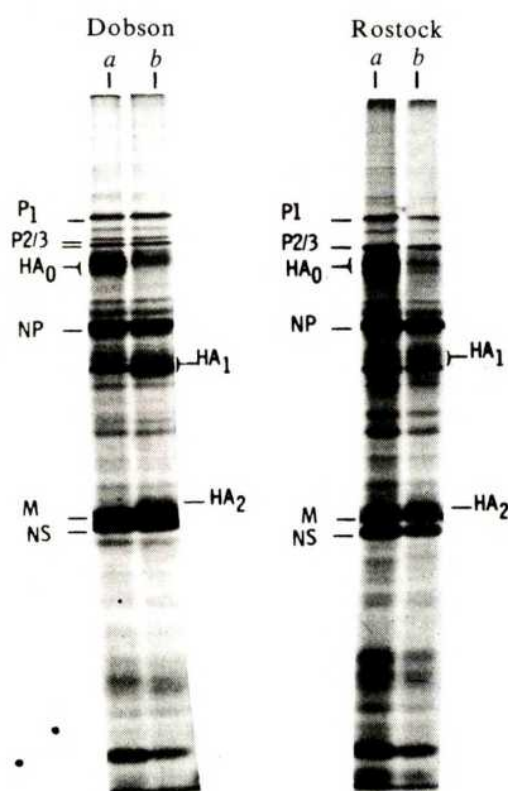
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**Fig. 1** Pulse chase experiments: Tracks (a) show FPV Rostock and FPV Dobson infected cell polypeptides synthesised during a 15-min pulse with <sup>35</sup>S-methionine at 4 h after infection. b, Similar samples which have been 'chased' for 45 min by adding medium containing cold methionine after removal of the label. Samples were prepared and subjected to polyacrylamide gel electrophoresis as described by Inglis *et al.*<sup>4</sup>.

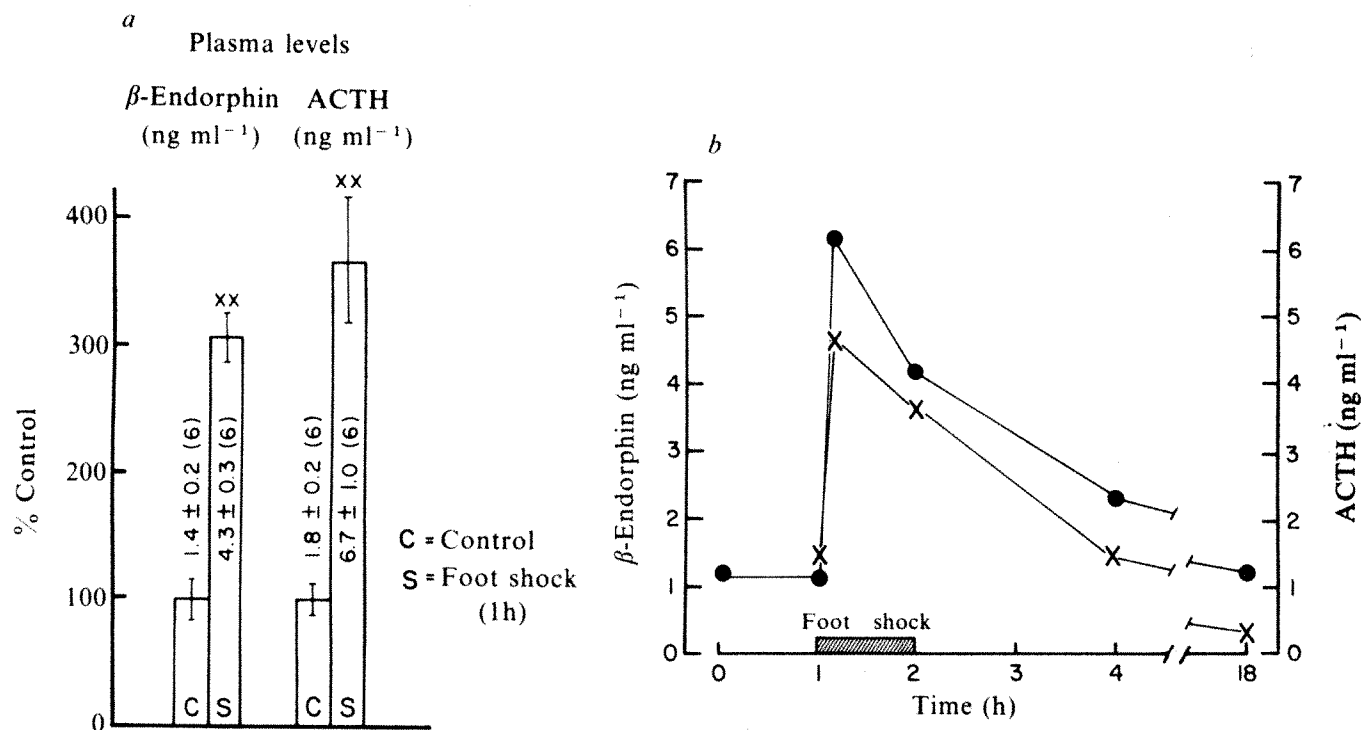


1. Pons, M. W. *Virology* **69**, 789-792 (1976).
2. Palese, P. & Schulman, J. L. *J. Virol.* **17**, 876-884 (1976).
3. McGeoch, D., Fellner, P. & Newton, C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3045-3049 (1976).
4. Inglis, S. C., Carroll, A. R., Lamb, R. A. & Mahy, B. W. J. *Virology* **74**, 489-503 (1976).
5. Palese, P. & Schulman, J. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2142-2146 (1976).
6. Palese, P., Ritchey, M. B. & Schulman, J. L. *Virology* **76**, 114-121 (1977).
7. Ritchey, M. B., Palese, P. & Schulman, J. L. *J. Virol.* **20**, 307-313 (1976).
8. Inglis, S. C., McGeoch, D. & Mahy, B. W. J. *Virology* **78**, 522-536 (1977).
9. Scholtissek, C., Harms, E., Rhode, W., Orlich, M. & Rott, R. *Virology* **74**, 332-344 (1976).
10. Skehel, J. J. *Virology* **49**, 23-36 (1972).
11. Almond, J. W., McGeoch, D. & Barry, R. D. *Virology* **81**, 62 (1977).
12. Ritchey, M. B., Palese, P. & Schulman, J. L. *Virology* **76**, 122-128 (1977).
13. Palese, P., Ritchey, M. B. & Schulman, J. L. *J. Virol.* **21**, 1187-1195 (1977).
14. Ritchey, M. B. & Palese, P. *J. Virol.* **21**, 1196-1204 (1977).
15. Burnet, F. M. & Lind, P. E. *Nature* **173**, 627 (1954).
16. Rott, R., Orlich, M. & Scholtissek, C. *J. Virol.* **19**, 54-60 (1976).
17. Staiger, H. R. *Virology* **22**, 419-422 (1964).
18. Tuckova, E., Vonka, V. & Starek, M. *Acta Virol.* **12**, 316-323 (1968).
19. Klenk, H. D., Rott, R., Orlich, M. & Blodorn, J. *Virology* **68**, 426-439 (1975).
20. Lazarowitz, S. G. & Choppin, P. W. *Virology* **68**, 440-454 (1975).
21. Lazarowitz, S. G., Compans, R. W. & Choppin, P. W. *Virology* **52**, 199-212 (1973).
22. Stanley, P., Gandhi, S. S. & White, D. O. *Virology* **53**, 92-106 (1973).
23. Ho, P. P. K., Young, A. L. & Truehaft, M. J. *gen. Virol.* **33**, 143-145 (1976).
24. Appleyard, G. & Maber, H. B. *J. gen. Virol.* **25**, 351-357 (1974).
25. Barry, R. D., Ives, D. R. & Cruickshank, J. G. *Nature* **194**, 1139-1140 (1962).
26. Compans, R. W. & Choppin, P. W. in *Comprehensive Virology* **4** (eds Fraenkel-Conrat, H. & Wagner, R. R.) 179-252 (1975).
27. Spooner, L. R. & Barry, R. D. *Nature* **268**, 650 (1977).

## Foot-shock induced stress increases $\beta$ -endorphin levels in blood but not brain

FOOT-SHOCK induced stress promotes a five to sixfold increase in  $\beta$ -endorphin plasma levels. Similar increases were also found for ACTH plasma levels. In the hypothalamus, foot-shock induced stress promotes a decrease of  $\beta$ -endorphin assayed by radioimmunoassays. These data suggest that physiological increases in plasma  $\beta$ -endorphin levels induced by stress do not result in elevated levels of brain  $\beta$ -endorphin.

Akil *et al.*<sup>1</sup> have shown that acute stress induced by inescapable



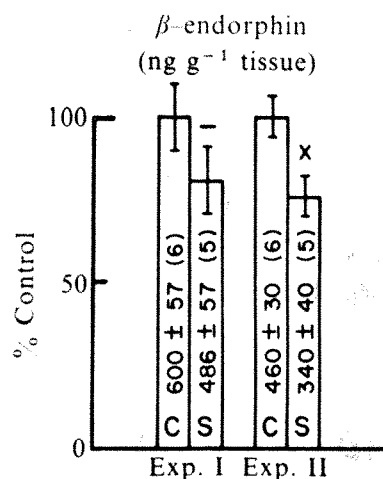
**Fig. 1** *a*, Stress and plasma levels of ACTH and  $\beta$ -endorphin. Rats (Sprague Dawley, male 200 g) were stressed by exposure for 1 h to inescapable electric foot shocks (1 mA, 1 s duration, at random 12 per min). Directly after the end of the stress period, they were decapitated and the trunk blood was collected on EDTA. After immediate centrifugation, the plasma was frozen until RIA. Randomised controls from the same lot of animals were killed at the same time (10 a.m.) directly after their removal from their cages. Their plasma samples were processed together with the samples of the stressed animals. *b*, Time course study of blood levels for ACTH (×) and  $\beta$ -endorphin (●) after stress. A jugular cannula was implanted in the right jugular of a rat (Sprague Dawley, male 200 g). The cannula was flushed each day with a solution of heparin. Three days after the surgery, the stress experiment as described in Fig. 1*a* was performed. The rat was placed in the experimental Perspex box one hour before the first electric foot shock. Blood samples were drawn 1 h and 1 min before the first shock and at the subsequent times indicated and were processed as described above.

electric foot shock promotes analgesia in rats and that this analgesic effect of stress was partially reversed by naloxone, a morphine antagonist. Simultaneously with that report, the explosion of observations began which characterised the endogenous brain peptides with opioid activity enkephalins<sup>2,3</sup> and endorphins<sup>4,6</sup>. Therefore, it was tempting to seek correlations between the stress-induced analgesia and the levels of the brain opioid peptides. Madden *et al.*<sup>7</sup> reported that levels of opioid brain materials were increased after stress. That study, however, used radio-receptor displacement assays which can not distinguish among the several endogenous peptides which share this biological property *in vitro*. Therefore, it was revealing when Fratta *et al.*<sup>8</sup> using a radioimmunoassay for Met<sup>5</sup>-enkephalin, were unable to detect any increase in brain immunoreactive material after stress. However, they proposed that another opioid peptide,  $\beta$ -endorphin, could have been mobilised from pituitary by stress to accumulate in brain tissue.

We have observed here that which  $\beta$ -endorphin is mobilised from the pituitary during stress and accumulates up to sixfold in plasma, the released  $\beta$ -endorphin does not accumulate in brain. On the contrary, hypothalamic levels of  $\beta$ -endorphin are slightly, but significantly reduced after the same 30 min episode of electric foot-shock stress.

Double antibody radioimmunoassay (RIA) for  $\beta$ -endorphin was previously described<sup>9,10</sup>. The RIA is specific to the Leu<sup>14</sup>-His<sup>27</sup> segment<sup>10</sup>. As  $\beta$ -endorphin is the C-terminal 31 amino acid fragment of  $\beta$ -lipotropin, this RIA could reflect any  $\beta$ -lipotropin in our samples. The 31,000 MW pre-prohormone isolated from clonal cultures of mouse adenohypophyseal tumour cells can also be read in this  $\beta$ -endorphin RIA<sup>11</sup>. Nevertheless, gel filtration sizing experiments have shown that the  $\beta$ -endorphin immunoreactivity in our samples (plasma and hypothalamus extracts) was associated mainly with a compound eluting in a pattern non-distinguishable from the one of synthetic  $\beta$ -endorphin<sup>12</sup>. ACTH RIA was performed using a procedure previously described<sup>13</sup>.

Two series of rats (Sprague Dawley, male, 200 g) were stressed by inescapable electric foot shock (1 mA, 1 s duration, at random 12 shocks per min) during 30 min or 1 h. Such treatment clearly produces stress as shown by a more than three-fold increase in both  $\beta$ -endorphin and ACTH plasma concentration (Fig. 1*a*). The peak of the stress-induced secretion was found 10 min (Fig.



**Fig. 2** Hypothalamus content in opioid peptides after acute stress. Two stress protocols were used. In experiment I (Expt I) animals were stressed exactly as in the protocol described in Fig. 1*a*. In experiment II (Expt II) the foot shocks were given for only 30 min and after a delay of 15 min after the shocks the animals were killed. In both experiments, brains were quickly removed and dissected as outlined by Glowinski & Iversen<sup>14</sup>. The tissues were frozen on dry ice, weighed and poured in 2 ml of 1 M acetic acid preheated to 98 °C. After 15 min in the boiling bath, the tissues were homogenised (polytron, setting 6, 10 s). After centrifugation (1,000g for 1 h) the supernatant was neutralised with 1 M NaOH and frozen before RIA. Results are expressed in ng per g of tissue.

1b) after the start of the foot shocks for both ACTH and  $\beta$ -endorphin which are secreted concomitantly in equimolar amounts, as previously reported<sup>14</sup>. Brain levels of  $\beta$ -endorphin were measured after 1 h of stress. No significant differences were observed in any brain region except in hypothalamus where the concentration of  $\beta$ -endorphin immunoreactivity was decreased. This difference, however, was not statistically significant. The stress experiment was then repeated, using a protocol identical to that of Akil *et al.*<sup>1</sup> consisting of foot shock for 30 min followed by a delay of 15 min before killing. With this protocol, again, a decrease in  $\beta$ -endorphin immunoreactivity was observed only in hypothalamus, and this difference was now statistically significant. No difference was observed in the other parts of the brain, pituitary, or pineal.

Stress or acupuncture stimulation are reported to induce analgesia which is in part reversed by naloxone<sup>1,15,16</sup>. Our results here indicate that while the analgesia may be concurrent with an increase of the secretion of  $\beta$ -endorphin from the pituitary into blood, the brain content of  $\beta$ -endorphin shows decreased content in hypothalamus and no change elsewhere. This decrease could most simply reflect the net effect of the release and degradation of the  $\beta$ -endorphin peptide from hypothalamic nerve processes. Indeed  $\beta$ -endorphin is stored like a neurotransmitter in neurones (ref. 12 and to be published). Nevertheless, we cannot with confidence correlate our findings with the mechanisms involved in pain suppression. Although intravenous infusion of  $\beta$ -endorphin induces analgesia in mice at doses of 8 mg kg<sup>-1</sup> or more<sup>17,18</sup>, we find no analgesia in rats after even higher doses. Also, the blood concentrations reached by such doses would be more than three orders of magnitude higher than the sixfold changes observed after the physiological shifts with stress observed here. Further work is required to establish whether endocrine secretion of pituitary  $\beta$ -endorphin and ACTH are causally related to the central analgesia produced by stress or whether hypothalamic  $\beta$ -endorphin content is depleted as a result of the behavioural disturbances evoked by our catastrophic stimuli. The present observations suggest, however, that increased blood levels of  $\beta$ -endorphin are not reflected by increases in brain  $\beta$ -endorphin.

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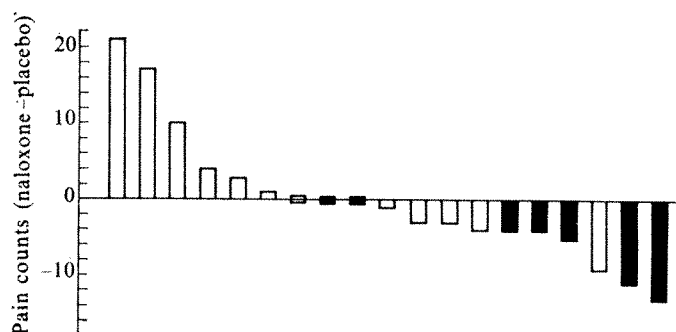
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1. Akil, H., Madden, J. IV, Patrick, R. L. & Barchas, J. D. in *Opiates and Endogenous opioid peptides* (ed. Kosterlitz, H. W.) 63-70 (Elsevier, Amsterdam, 1976).
2. Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. & Morris, H. R. *Nature* **258**, 577-579 (1975).
3. Simantov, R. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2515-2519 (1976).
4. Guillemin, R., Ling, N. & Burgus, R. C. *r. heb. Séanc. Acad. Sci. Paris D* **282**, 783-785 (1976).
5. Cox, B. M., Goldstein, A. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1821-1823 (1976).
6. Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M. & Holme, E. C. *Nature* **260**, 793-795 (1976).
7. Madden, J. IV, Akil, H., Patrick, R. L. & Barchas, J. D. *Nature* **265**, 358-360 (1977).
8. Fratta, W., Yang, H.-Y. T., Hong, J. & Costa, E. *Nature* **268**, 452-453 (1977).
9. Rossier, J., Bayon, A., Vargo, T. M., Ling, N., Guillemin, R. & Bloom, F. E. *Life Sci.* **21**, 847-852 (1977).
10. Guillemin, R., Ling, N. & Vargo, T. M. *Biochem. Biophys. Res. Commun.* **77**, 361-366 (1977).
11. Mains, R., Eipper, E., Ling, N. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3014-3018 (1977).
12. Rossier, J., Vargo, T. M., Minick, S., Ling, N., Bloom, F. E. & Guillemin, R. *Proc. natn. Acad. Sci. U.S.A.* (in press).
13. Madden, J. IV, Rivier, C. *Fedn Proc.* **36**, 2094-2099 (1977).
14. Guillemin, R., Vargo, T. M., Rossier, J., Minick, S., Ling, N., Rivier, C. & Bloom, F. E. *Science* **197**, 1367-1369 (1977).
15. Pomeranz, B. & Chiu, D. *Life Sci.* **19**, 1757-1762 (1976).
16. Mayer, D. J., Price, D. D. & Rafii, A. *Brain Res.* **121**, 368-372 (1977).
17. Graf, L., Szekely, J. I., Ronai, A. Z., Dunai-Kovacs, Z. & Bajusz, S. *Nature* **263**, 240-242 (1976).
18. Loh, H. H., Tseng, L. F., Wei, E. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2895-2898 (1976).
19. Glowinski, J. & Iversen, L. L. *J. Neurochem.* **13**, 655-669 (1966).

## Naloxone alters pain perception and somatosensory evoked potentials in normal subjects

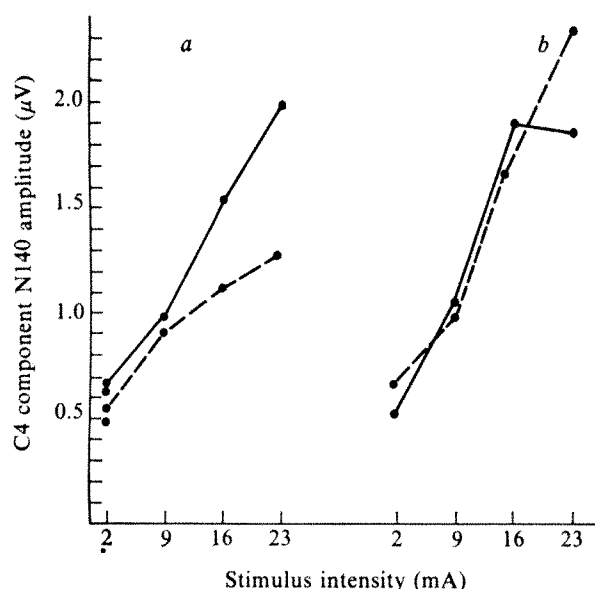
THE discovery of opiate-like peptides (endorphins) in brain, blood and cerebrospinal fluid<sup>1-4</sup> raises questions as to the physiological role of these substances. Given the prominent analgesic effects of opiates, the regulation of pain perception is a candidate for endorphin action. Endorphins microinjected into the periaqueductal grey matter of rats induce analgesia which is reversed by naloxone, a pure narcotic antagonist<sup>5</sup>. If endorphins play an active part in the regulation of pain, then naloxone administered to man should alter pain appreciation. Naloxone has not been shown to have analgesic, respiratory, euphoric, pupillary or electroencephalogram (EEG) effects in man<sup>6</sup>. Studies in rats<sup>7,8</sup> and one early study in man<sup>9</sup> have suggested slight hyperalgesic effects with naloxone. El-Sobky *et al.*<sup>10</sup> failed to demonstrate naloxone effects on electric shock pain judgments in five subjects. In the experiments reported here, subjects were divided into pain sensitive and pain insensitive subgroups. The insensitive subjects found shocks significantly more painful after naloxone administration while the sensitive group experienced them as less painful. Evoked potentials showed similar significant group differences. These results suggest that individual differences in pain sensitivity may relate to differences in an endorphin system.

Normal adult volunteers (10 male, 11 female; mean age 20 yr) gave informed consent and participated in three similar experimental sessions. Each session consisted of two psychophysiological pain rating tests and a pain somatosensory evoked potential (EP) test. The initial session was for familiarisation and no drug was given. On the following two sessions the subjects either received an intravenous injection of 2.0 mg naloxone (0.4 mg ml<sup>-1</sup>) or an equal volume of saline (0.9%) in a random order and double-blind fashion. Experimental procedures began 5 min after injection and lasted 20 min. All procedures involved single shocks administered to the left forearm by a computer-controlled constant current stimulator with a Tursky electrode<sup>11</sup>. Subjects received three shocks at each milliamperage increment from 1 to 31 mA for a total of 93 shocks in a random sequence at 2.5-s intervals. Subjects rated each shock in one of four categories; noticeable, distinct, unpleasant or very unpleasant. Signal detection analysis yielded two pain measures: a nonparametric analogue of response criterion and a sensitivity level<sup>12</sup>. The sensitivity measure has been associated with pharmacological analgesia<sup>14</sup> and response criterion related to suggestion effects<sup>15</sup>.



**Fig. 1** Pain counts (unpleasant plus very unpleasant subjective ratings) illustrated as the difference between naloxone and placebo days for each individual studied. Positive differences thus indicate more stimuli rated as unpleasant or worse on naloxone than on placebo. Subjects were divided into pain-insensitive and pain-sensitive groups on the basis of a sensitivity measure derived from signal detection analysis during a separate pre-drug trial session. Note that no pain-sensitive subject became more sensitive on naloxone while most pain-insensitive individuals did (groups significantly different by *t* test on individual difference scores  $P < 0.05$ ). Two subjects were excluded because no stimulus was rated above distinct on the familiarisation day and thus the sensitivity could not be calculated. Open bars, pain-insensitive subjects; solid bars, pain-sensitive subjects.





**Fig. 2** Mean evoked potential amplitude in  $\mu\text{V}$  for negative peak at about 140 ms at four stimulus intensities for pain sensitive and insensitive subject groups. Note EP differences at highest stimulus intensities. Data were analysed using a three-way analysis of variance with repeated measures for the intensity and drug dimensions and independent groups for the third dimension; linear trend analysis was used on the intensity dimension. Group differences were statistically confirmed (group  $\times$  drug  $\times$  intensity interaction,  $F = 4.45$ , 1, 17 d.f.  $P < 0.05$ ). EP differences between sensitive and insensitive groups on placebo can also be seen. *a*, Pain-insensitive group. *b*, Pain-sensitive group. Solid lines, placebo; broken lines, naloxone.

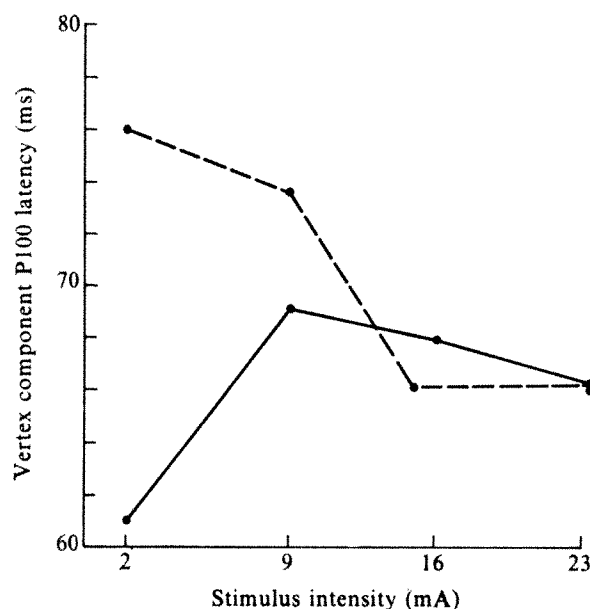
In addition, we computed the mean amperage associated with each stimulus category and we counted the number of responses out of the total of 93 judged unpleasant and very unpleasant (termed 'pain counts'). The evoked potential procedure used four stimulus levels (2, 9, 16, and 23 mA) presented 64 times at 1-s intervals in a random order; EEG was recorded from vertex and somatosensory cortex (C4) and averaged separately for each intensity<sup>13</sup>. Evoked potential (EP) amplitude was measured for the three major EP components, a positive P100 (76–112 ms), a negative N140 (116–152 ms) and a positive P200 (168–248 ms)<sup>13</sup>.

Subjects were divided into pain sensitive and pain insensitive groups based on whether their pain sensitivity level on the familiarisation day fell above or below the mean of subjects used in two previous pain studies<sup>12,13</sup>. Individuals so defined as pain insensitive showed naloxone hyperalgesia. They rated stimuli of lower milliamperage as unpleasant and judged a higher number of the 93 stimuli as unpleasant after naloxone administration, whereas pain sensitive individuals showed the opposite effect (Fig. 1). Familiarisation session sensitivity significantly predicted 'pain count' changes between placebo and naloxone ( $r = 0.49$ ,  $P < 0.05$ ). While both the sensitivity and the response criterion measures similarly indicated greater naloxone hyperalgesia in the pain insensitive group, neither measure reached statistical significance. But, the distinct/unpleasant response criterion determined for the familiarisation session did predict the change by naloxone in the mean milliamperage for the very unpleasant category ( $r = -0.47$ ,  $P < 0.05$ ).

Similar group differences were seen for EP results (Fig. 2). Peak N140 amplitude for the vertex lead increased following naloxone by 0.36  $\mu\text{V}$  in the insensitive group but decreased in the sensitive group (group by drug interaction,  $F = 4.47$ , 1, 17 d.f.  $P < 0.05$ ). In EPs recorded from C4, this effect was especially pronounced at the highest shock intensities. EP latency was also significantly shorter after naloxone in the insensitive group especially at low intensities for P100, N140 and P200 (analysis of variance on each of three peaks,  $P < 0.05$ , group by intensity by drug interaction). The sensitive group showed increases in latency at low intensity

for all three peaks. The groups as divided on the pain sensitivity measure also differed in the rate of increase in EP amplitude with increasing stimulus intensity of the C4 P100 component; pain insensitive individuals showing lower amplitude/intensity slopes ( $F = 4.27$ , linear trend effect  $P < 0.05$ ). This pattern has been referred to as 'reducing' and previously reported in other pain insensitive groups<sup>16</sup>.

When subjects were examined as a single group (Fig. 3) the P100 latency for the lowest intensities was significantly decreased following naloxone. The N140 component of the EP also demonstrated shorter latency for all subjects after naloxone ( $F = 5.17$ ,  $P < 0.05$ ) with no effect in P200 latency. Neither sensitivity nor response criterion measures for the distinct/unpleasant division were significantly different between drug conditions. Naloxone administration also failed to change the mean EP amplitude across intensities or the amplitude/intensity slope for P100, N140 and P200.



**Fig. 3** Mean evoked potential latency in ms for the positive component at about 100-ms post-stimulus for naloxone and placebo conditions for all 21 subjects. This effect was statistically confirmed by 2-way ANOVA, ( $F = 6.41$ , drug by intensity interaction, 1, 20 d.f., linear trend effect,  $P < 0.05$ ). Solid line, naloxone; broken line, placebo treatment.

Our finding of naloxone effects on pain response supports the hypothesis that the endogenous opiate-like substances have a physiological role in pain regulation. The finding of group differences both in EPs and in subjective ratings is not merely a statistical artefact as a totally independent session was used to categorise the subjects, and as both groups changed it does not seem to reflect only response ceiling or floor effects. Individuals with relatively higher levels of endorphins, relatively more opiate receptors or relatively more highly developed pain inhibitory pathways might be more naloxone responsive. If these factors were the only source of individual variation, however, one might have expected to see only the pain insensitive group respond to naloxone, rather than the bidirectional effects observed. Lasagna<sup>9</sup> noted a "strange biphasic quality" of naloxone response with 2 mg apparently causing analgesia but higher doses having hyperalgesic effects. Similarly supporting bidirectionality, Leybin *et al.*<sup>17</sup> found an unexpected increase in the perception of pain in rats given the endogenous opiate Met-enkephalin. Bidirectional effects may reflect individual differences in the functional activity of complementary pain modulation systems, rather than a dose dependent effect at receptor sites. A primary analgesic action of endogenous opiate-like substances similar to the actions of

exogenous opiates is not clearly supported by our data. The bidirectional effects observed here and elsewhere suggest a modulatory rather than strictly analgesic role for endorphins.

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1. Hughes, J. *Brain Res.* **88**, 259–308 (1975).
2. Terenius, L. & Wahlstrom, A. *Life Sci.* **16**, 1759–1764 (1975).
3. Frederickson, R., Schirmer, E. W., Grinnan, E. L., Harrell, C. W. & Hewes, C. R. *Life Sci.* **19**, 1181–1184 (1976).
4. Pert, C. B., Pert, A. & Tallman, J. F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2226–2230 (1976).
5. Pert, A. in *Opiates and Endogenous Opioid Peptides* 87–94 (Elsevier, North Holland, New York, 1976).
6. Jasinski, D. R., Martin, W. R. & Haertzen, C. A. *J. Pharmac. exp. Ther.* **157**, 420–426 (1967).
7. Jacob, J. J., Tremblay, E. C. & Colombel, M. C. *Psychopharmacologia (Berl.)* **37**, 217–223 (1974).
8. Frederickson, R. C. A., Nickander, R., Smithwick, E. C., Shuman, R. & Norris, F. H. in *Opiates and Endogenous Opioid Peptides* 239–246 (Elsevier North Holland, New York, 1976).
9. Lasagna, L. *Proc. R. Soc. Med.* **58**, 978–983 (1965).
10. El-Sobky, A., Dostrovsky, J. D. & Wall, P. D. *Nature* **263**, 783–784 (1976).
11. Tursky, B. *Psychophysiology* **11**, 95–112 (1974).
12. Sitaram, N., Buchsbaum, M. S. & Gillin, J. C. *Eur. J. Pharm.* **42**, 285–290 (1977).
13. Lavine, R., Buchsbaum, M. S. & Poncy, M. *Psychophysiology* **13**, 140–148 (1976).
14. Chapman, C. R., Murphy, T. M. & Butler, S. H. *Science* **179**, 1246–1248 (1972).
15. Clark, W. C. & Goodman, J. S. *J. Abnormal Psych.* **83**, 364–372 (1974).
16. Buchsbaum, M. S. in *Biology of the Major Psychoses: A comparative Analysis* 129–142 (Raven, New York, 1975).
17. Leybin, L., Pinsky, C., Labella, F. S., Havelicek, V. & Rezek, M. *Nature* **264**, 458–459 (1976).

## Do snail neurones contain more than one neurotransmitter?

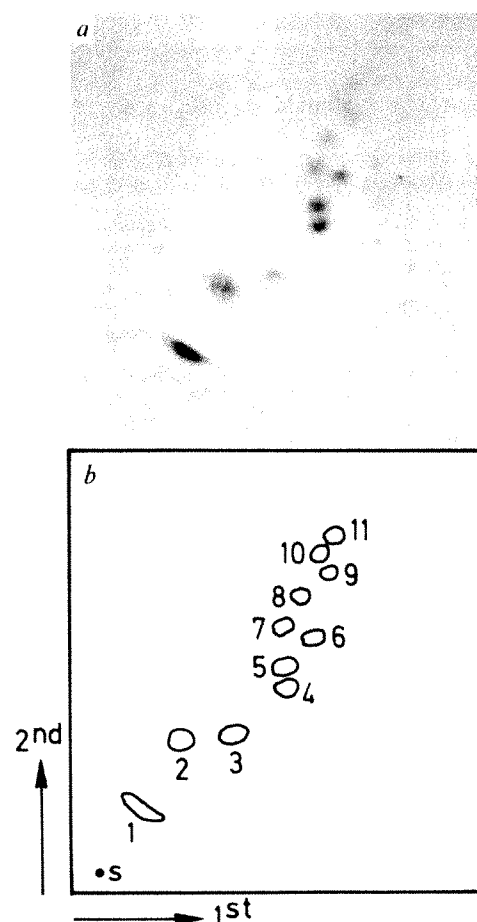
THE 5-hydroxytryptamine (5-HT) present in the giant neurone in each metacerebral ganglion (GSC) of the snail, *Helix pomatia*, is probably a transmitter<sup>1–3</sup>. The neurone also contains acetylcholine<sup>4</sup>, a putative transmitter and its synthesising enzyme, choline acetyltransferase (Ch.Ac., EC 2.3.1.6) (ref. 5). These observations, together with a report showing multiple putative transmitter substances in certain *Aplysia* neurones<sup>6</sup>, have resulted in several challenges<sup>2,6,7</sup> of the validity of Dale's hypothesis that each neurone contains and releases only one transmitter. The GSC is found in other gastropods, in every case containing 5-HT (refs 9,10); there is high Ch.Ac. activity in *Helix*<sup>5</sup>, but in *Aplysia* the enzyme is absent<sup>11</sup> or present in trace amounts<sup>8</sup>. All previous work on *Helix* neurones has been carried out on hand-dissected GSCs, and so contamination from glial and other small neurones must always be considered (see ref. 12). If a neurone is considered to be spherical, a slight increase in the radius would result in a substantial volume of substance being trapped between the neuronal membrane and the contaminating membrane. The isolation process is thus of critical importance. I have reanalysed the neurone for a spectrum of transmitters to investigate whether the GSCs can synthesise 'common' transmitter substances. A neurone-designated cell 21<sup>2,5,13</sup>, situated on the posterior–dorsal surface of the visceral ganglia and known to contain high levels of Ch.Ac. was also examined for comparison. I show that the GSC contains only 5-HT, with no significant Ch.Ac., and that it also contains trace amounts of dopamine and histamine.

Single neurones were dissected from fresh snails (*Helix pomatia*) caught locally and the isolated perikarya were freed from adhering tissue with special care<sup>12</sup>. Four neurones of each type were placed in 10- $\mu$ l capillary tubes containing snail physiological saline<sup>14</sup> and then washed thoroughly by centrifugation and removal of the supernatant. This process was repeated and the neurones analysed for their histamine<sup>15</sup>, octopamine<sup>16</sup>, dopamine and noradrenaline<sup>17</sup>, Ch.Ac. (ref. 18), 5-HT (refs 12, 19, 20) and amino acid content<sup>12,19</sup>.

A second series of experiments tested the ability of the two neurone types to synthesise transmitters. Circumoesophageal ganglia were incubated in 1 ml of snail saline<sup>14</sup> with 30  $\mu$ M of previously purified <sup>3</sup>H-precursors (all with specific activities of 12–23 Ci mmol<sup>-1</sup>) to transmitters for 60 min at 26 °C and individual cells dissected<sup>12</sup>. Groups of four cells were washed twice with physiological saline and homogenised in their capillary tubes in 50% acetone containing 0.1 N HCl. After centrifugation,

the supernatants were analysed by two-dimensional chromatography on silica gel plates for amine transmitters (see Fig. 1) or by one-dimensional chromatography on cellulose plates for acetylcholine (see Table 2).

The results show clearly (Table 1) that the only putative transmitter present in unique and high concentrations in the GSC is 5-HT, thus differing from the report of Hanley *et al.*<sup>5</sup> that the neurone also contains high amounts of Ch.Ac. The assay conditions for Ch.Ac. might not have been optimal, but this was taken into consideration. Moreover, the activity of Ch.Ac. found in cell 21 is of the same order as that found by Hanley *et al.*<sup>5</sup>. The GSCs also contain trace amounts of histamine and dopamine, as does cell 21, which also has a minute amount of 5-HT. Since it is impossible to dissect an absolutely 'clean' neurone because of glial investment, adhering satellite cells and exogenous substances released from other damaged cells<sup>12</sup>, and since highly sensitive assay procedures are used, the background noise due to impurities



**Fig. 1** *a*, A chromatogram (precoated silica gel 5 × 5 cm) and *b*, a corresponding map to show the relative positions of various transmitter substances and precursors separated in two solvent systems. The directions of chromatography are indicated by the arrows and the solvent systems used were butanol–acetic acid–water (15:3:5 by vol.) in the first direction and butanol–pyridine–acetic acid–water (15:2:3:5 by vol.) in the second direction. The substances were visualised on the chromatograms by initial exposure to paraformaldehyde vapour for 30 min at 80 °C followed by spraying with 1% potassium ferricyanide in ammonium hydroxide solution. After drying, the substances were sprayed with ninhydrin solution. S, Starting point; 1, glutamate; 2, GABA; 3, DOPA; 4, 5-hydroxytryptophan; 5, tyrosine; 6, tryptophan; 7, noradrenaline; 8, dopamine; 9, 5-hydroxytryptamine; 10, octopamine; 11, tyramine. Individual spots were then removed from the chromatogram, the substance was eluted with methanol and the amount calculated by scintillation spectrometry and, assuming the specific activity of the products to be equal to the <sup>3</sup>H-precursor, added to the ganglia at the start of the incubation. Recovery of all tritiated substances was in the range of 60–80%. Cell 21 did not metabolise <sup>3</sup>H-tyrosine, <sup>3</sup>H-tryptophan or <sup>3</sup>H-tyramine but the GSCs formed <sup>3</sup>H-5-hydroxytryptophan and <sup>3</sup>H-5-HT from the <sup>3</sup>H-tryptophan. The possibility of the formation of <sup>3</sup>H-GABA from glutamate was not tested, since whole brain tissue cannot form GABA.

**Table 1** Transmitter candidates in GSCs and cell 21 of *Helix pomatia*

Substance	Concentration in the GSC	Concentration in cell 21	Procedure used (ref.) and sensitivity actually achieved
5-Hydroxytryptamine	$3.8 \times 10^{-4}$ M	$2 \times 10^{-8}$ M	ref. 20 500 pg and dansyl method <sup>19</sup> 100 pg
Histamine	$2 \times 10^{-8}$ M	$10^{-8}$ M	ref. 15 100 pg
Octopamine	N.D.	N.D.	ref. 16 50 pg
Dopamine	$8 \times 10^{-9}$ M	$3 \times 10^{-8}$ M	ref. 17 50 pg
Noradrenaline	N.D.	N.D.	ref. 17 50 pg
Ch.Ac.	0.2 pmol per cell per h	61 pmol per cell per h	ref. 18 Blank corresponded to 1% of radioactivity in incubation medium
Glutamate	$6 \times 10^{-4}$ M	$3 \times 10^{-4}$ M	Dansyl method <sup>19</sup> 100 pg
Aspartate	$7 \times 10^{-5}$ M	$9 \times 10^{-5}$ M	
Glycine	$5 \times 10^{-4}$ M	$5.9 \times 10^{-4}$ M	
Taurine	$6 \times 10^{-6}$ M	$6 \times 10^{-6}$ M	

The total volume of each cell type was estimated by measuring the diameters of a number of cells (mean diameter of both cell types 120–140  $\mu$ m) under light microscopy. It was found that the GSC and cell 21 had volumes of 1.2 nl. The molarity (results reported for 5–7 determinations; in each experiment four neurones were pooled) of each substance in the two neurone types could then be calculated.

N.D. = not detected.

will tend to be amplified. It is therefore suggested that the trace amounts of histamine, dopamine and Ch.Ac. in the GSC are artefacts. Interesting in the original publication of Emson *et al.*<sup>13</sup> is a note describing the activity of Ch.Ac. in the GSCs as 'small', whereas in the later publication<sup>5</sup> the Ch.Ac. activity in the GSC is of the order of 21 pmol per cell per h. The concentration of the putative transmitter amino acids in both cell types was similar, suggesting that they do not have a transmitter function in these neurones.

incubation solution had no effect, substantiating previous data<sup>1</sup>.

These results suggest that the GSCs produce and contain one putative transmitter substance, 5-HT, and not ACh. Cell 21 seems, in contrast, to use only ACh. The question of whether multiple neurotransmitters exist in neurones<sup>4–8</sup> should therefore be reassessed critically, especially when the evidence is biochemical and drawn from hand-dissected neurones, where the degree of error may be magnified. Trace quantities of substances could be endogenous in origin, since from the evolutionary point of view,

**Table 2** <sup>3</sup>H-ACh and <sup>3</sup>H-5-HT formed from 30  $\mu$ M <sup>3</sup>H-choline or <sup>3</sup>H-tryptophan

	<sup>3</sup> H-ACh, fmol		<sup>3</sup> H-5-HT, fmol	
	Without eserine	With eserine	Without pargyline	With pargyline
per GSC	$0.2 \pm 0.2^\dagger$ (12)	$0.5 \pm 0.2^\dagger$ (12)	$9 \pm 0.9$ (5)	$9 \pm 1$ (5)
per cell 21	$18 \pm 3^\dagger$ (12)	$56 \pm 6^\dagger$ (12)	$0.2 \pm 0.2^*$ (5)	$0.2 \pm 0.2^*$ (5)
per cerebral ganglion	$3,000 \pm 105$ (3)	$4,900 \pm 195$ (3)	$71 \pm 4$ (3)	$69 \pm 5$ (3)

All values are  $\pm$  s.e.m. with the number of determinations (in each determination four neurones were pooled) in parentheses, and were calculated by taking into consideration the specific activity of the <sup>3</sup>H-choline and <sup>3</sup>H-tryptophan. <sup>3</sup>H-Amine neurotransmitters were fractionated from other substances by two-dimensional chromatography on 5  $\times$  5-cm precoated silica gel plates (see Fig. 1) while <sup>3</sup>H-ACh was separated from <sup>3</sup>H-choline by single-dimensional chromatography on 5  $\times$  5-cm precoated cellulose plates using the solvent system butanol-ethanol-acetic acid-water (8:2:1:3 by vol.). <sup>3</sup>H-ACh was visualised by iodine vapour and eluted from the chromatogram with methanol, and the absolute amount of <sup>3</sup>H-ACh calculated by assuming the specific activity to be equal to <sup>3</sup>H-choline added to the ganglia at the start of the incubation. Recovery of <sup>3</sup>H-choline and <sup>3</sup>H-acetylcholine was 85%.

\*Not significantly different from blank values (Student's *t* test  $P > 0.05$ ).

$^\dagger$ Significantly different from experiments without eserine and with blank values (Student's *t* test  $P < 0.05$ ).

The results of the second series of experiments supported the first. Initial experiments showed that snail ganglia can synthesise <sup>3</sup>H-ACh, <sup>3</sup>H-5-HT, <sup>3</sup>H-dopamine and <sup>3</sup>H-octopamine from their precursors, but not <sup>3</sup>H-GABA or <sup>3</sup>H-noradrenaline, thus confirming previous results<sup>3</sup>. The amount of <sup>3</sup>H-ACh formed was approximately 50-fold that of any other <sup>3</sup>H-transmitter. Cerebral ganglia contained  $\sim 3$  pmol of <sup>3</sup>H-ACh per ganglia after incubation with 30  $\mu$ M <sup>3</sup>H-choline (Table 2), but only 0.07 pmol of <sup>3</sup>H-5-HT, 0.06 pmol <sup>3</sup>H-dopamine or 0.03 pmol <sup>3</sup>H-octopamine following incubation with 30  $\mu$ M <sup>3</sup>H-tryptophan, <sup>3</sup>H-tyrosine or <sup>3</sup>H-tyramine. The amount of <sup>3</sup>H-ACh formed was further increased when incubated with  $10^{-4}$  M eserine (see Table 2). This study showed that small amounts of <sup>3</sup>H-transmitters could be detected in specific neurones, especially if the cells produced ACh. In fact, <sup>3</sup>H-ACh was readily detected in extracts of four isolated cell 21 neurones removed from ganglia previously incubated with <sup>3</sup>H-choline (Table 2). In contrast, only a trace amount of <sup>3</sup>H-ACh was found in extracts of either four or eight pooled GSCs. Since the limit of resolution of these experiments was of the order of 0.3 fmol of <sup>3</sup>H-ACh, about 2% of the level in a single cell 21, the inability to detect <sup>3</sup>H-ACh in the GSCs is probably not due to a lack of precursor, but to the absence of the synthetic enzyme. Inclusion of eserine in the incubations (Table 2) produced a very small but statistically detectable quantity of <sup>3</sup>H-ACh in the GSCs, and the amount of <sup>3</sup>H-ACh in the cell 21 was increased by a factor of 3.

The only other transmitter synthesised by the neurones was <sup>3</sup>H-5-HT which was limited to the GSCs, producing about 9 fmol <sup>3</sup>H-5-HT. A monoamine oxidase inhibitor (pargyline  $10^{-4}$  M) in the

all neurones are expected to contain the same genetic machinery. It will then remain to demonstrate that they are of physiological significance<sup>8</sup> and ask why neurones, which use the same transmitter substance, nevertheless, metabolise 100-fold different concentrations of the substance.

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- Osborne, N. N. in *Biochemistry of Characterised Neurons* (ed. N. N. Osborne) (Pergamon, Oxford, in the press).
- Cottrell, G. A. *Neuroscience* **2**, 1–18 (1977).
- Gerschenfeld, H. M. *Physiol. Rev.* **53**, 1–119 (1973).
- Hanley, M. R. & Cottrell, G. A. *J. Pharm. Pharmacol.* **26**, 980 (1974).
- Hanley, M. R., Cottrell, G. A., Emson, P. C. & Fonnum, F. *Nature* **251**, 631–633 (1974).
- Brownstein, M. J., Saavedra, J. M., Axelrod, J., Zeman, G. H. & Carpenter, D. O. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4662–4665 (1974).
- Burnstock, G. *Neuroscience* **1**, 239–248 (1976).
- McCaman, R. E. & McCaman, M. W. in *Biology of Cholinergic Function* 485–513 (Raven, New York, 1976).
- Osborne, N. N. & Cottrell, G. A. *Z. Zellforsch.* **112**, 15–30 (1971).
- Kehoe, J. & Marder, E. *Rev. Pharmacol. Toxicol.* **16**, 245–268 (1976).
- Weinreich, D., McCaman, M. W., McCaman, R. E. & Vaughn, J. E. *J. Neurochem.* **20**, 969–976 (1973).
- Osborne, N. N. in *Microchemical Analysis of Nervous Tissue* (Pergamon, Oxford, 1974).
- Emson, P. C. & Fonnum, F. *J. Neurochem.* **22**, 1079–1088 (1972).
- Meng, K. *Zool. Fähr.* **68**, 193–204 (1970).
- Snyder, S. H., Baldessarini, R. & Axelrod, J. *J. Pharmacol. exp. Ther.* **154**, 549–554 (1966).
- Molinoff, P. C., Landsberg, L. & Axelrod, J. *J. Pharmacol. exp. Ther.* **170**, 253–261 (1969).
- Palkovits, M., Brownstein, M. J., Saavedra, J. M. & Axelrod, J. *Brain Res.* **77**, 137–149 (1974).
- Fonnum, F. *J. Neurochem.* **24**, 407–410 (1975).
- Osborne, N. N. in *Progress in Neurobiology* **1** (eds Kerkut, G. A. & Phillis, J. W.) 301–332 (Pergamon, Oxford, 1973).
- McCaman, M. W., Weinreich, D. & McCaman, R. E. *Brain Res.* **53**, 129–137 (1973).



## $\alpha$ adrenoreceptors but not $\beta$ adrenoreceptors increase in rabbit uterus with oestrogen

STEROID hormones alter sympathetic function and modulate responses to catecholamines<sup>1-3</sup>. The gonadal steroids, oestrogen and progesterone, can affect sympathetic response by altering catecholamine metabolism, and, in addition, they produce qualitative changes in the contractile response of smooth muscle to stimulation by adrenaline and noradrenaline<sup>4-6</sup>. The response of human oviduct and uteri from several species changes from contraction to relaxation depending upon the concentrations of gonadal steroids. Contraction, which is mediated by  $\alpha$ -adrenergic receptors, is observed in uteri from oestrogen-treated humans or rabbits, while relaxation, a  $\beta$ -adrenergic response, predominates during pregnancy or with progesterone treatment. Conversion between  $\alpha$  and  $\beta$  response, depending on hormonal or other environmental factors, has prompted the hypothesis that  $\alpha$ - and  $\beta$ -adrenergic receptors may be interconvertible<sup>7-9</sup>. We used the radioligands <sup>3</sup>H-dihydroergocryptine (DHE) and <sup>125</sup>I-iodohydroxybenzylpindolol ([<sup>125</sup>I]IHYP) to quantitate  $\alpha$ - and  $\beta$ -adrenergic receptors respectively in subcellular preparations of uteri from rabbits treated with oestrogen or oestrogen followed by progesterone.  $\alpha$ -Adrenergic binding sites were three times greater in uteri from the oestrogen-treated animals in which  $\alpha$ -adrenergic response was predominant than in uteri from animals treated with oestrogen followed by progesterone in which  $\beta$ -adrenergic response predominates. The number of  $\beta$ -adrenergic binding sites was unchanged by the different treatments and these sites were only 5% as numerous as  $\alpha$ -adrenergic sites. These findings suggest that gonadal steroids may alter smooth muscle adrenergic response by alterations of adrenergic receptor number and do not support the hypothesis that  $\alpha$ - and  $\beta$ -adrenergic receptors are simply interconvertible.

After injecting immature New Zealand rabbits intramuscularly with 150  $\mu$ g estradiol benzoate for 4 d, we prepared myometrial strips and examined isometric muscle contractility *in vitro*. Such strips exhibit spontaneous contractile activity, and contractions are increased in force and

frequency when  $5 \times 10^{-6}$  M noradrenaline is added. The stimulatory effect is inhibited by the  $\alpha$ -adrenergic antagonist phentolamine ( $10^{-5}$  M). When the 4 d of oestrogen treatment is followed by 4 d of treatment intramuscularly with 10 mg d<sup>-1</sup> progesterone,  $5 \times 10^{-6}$  M noradrenaline reduces spontaneous myometrial activity. This inhibitory effect is blocked by the  $\beta$ -adrenergic antagonist propranolol ( $10^{-5}$  M).

We prepared a subcellular fraction of myometrium from the animals after these treatment courses and studied binding of radioligands for the  $\alpha$ - and  $\beta$ -adrenergic receptors under identical conditions. Dissociation constants ( $K_d$ ) and numbers of binding sites were determined in equilibrium binding experiments (Table 1). Data were normalised to DNA content to correct for hyperplasia and hypertrophy of the myometrium.

$\alpha$ -Adrenergic receptors were quantitated with the radioligand DHE. Williams *et al.*<sup>11</sup> have shown, and we have confirmed, that binding of this agent is compatible with interaction at an  $\alpha$ -adrenergic receptor site in rabbit myometrium.  $\beta$ -Adrenergic receptors were quantitated with the radioligand [<sup>125</sup>I]IHYP. [<sup>125</sup>I]IHYP, a potent  $\beta$ -adrenergic antagonist, binds to subcellular preparations of several cells in a manner compatible with interactions at the  $\beta$ -adrenergic receptor<sup>12-16</sup>. In subcellular preparations of rabbit myometrium the binding of [<sup>125</sup>I]IHYP shows high affinity ( $K_d = 2 \times 10^{-10}$  M), low capacity (71 fmol mg<sup>-1</sup> DNA), and is readily reversible ( $t_{1/2} = 5$  min). The binding is inhibited by  $\beta$ -adrenergic agonists and antagonists, the active (–)-isomers being 20 times more potent than the corresponding (+)-isomers. The rank order of potency of adrenergic agonists for competition with [<sup>125</sup>I]IHYP is isoproterenol > epinephrine > norepinephrine—identical to the order for inhibition of spontaneous uterine activity by these agents<sup>3</sup>.

Subcellular preparations of myometrium from uteri of oestrogen-treated rabbits in which  $\alpha$ -adrenergic response was predominant had three times as many DHE binding sites as did uteri in which  $\beta$ -adrenergic response was predominant (progesterone treatment). This change in the number of binding sites was not accompanied by a significant change in apparent affinity of the binding sites for the radioligand (Fig. 1). Control animals, which were un-

**Table 1** Dissociation constants and number of binding sites for DHE and [<sup>125</sup>I]IHYP in oestrogen- or progesterone-treated rabbits

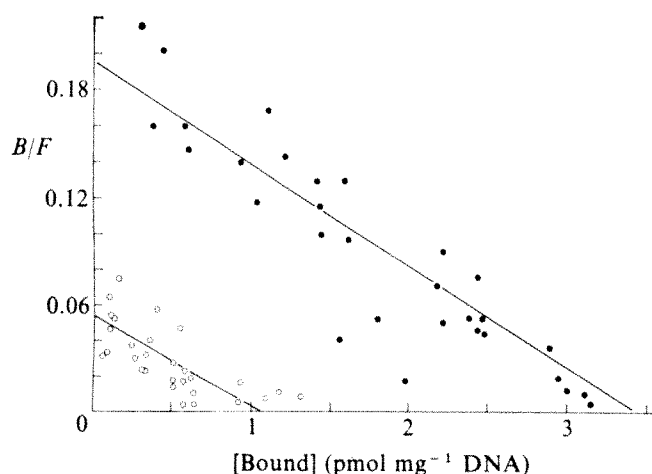
	DHE binding		[ <sup>125</sup> I]IHYP binding	
	Dissociation constant (nmol l <sup>-1</sup> )	Number of binding sites (fmol mg <sup>-1</sup> DNA)	Dissociation constant (nmol l <sup>-1</sup> )	Number of binding sites (fmol mg <sup>-1</sup> DNA)
Oestrogen	5.1 ± 1.1	3,400 ± 400	0.21 ± 0.03	86 ± 11
Progesterone	8.0 ± 2.8†	1,000 ± 200*	0.22 ± 0.02†	56 ± 9†

\*  $P < 0.001$ .

†  $P > 0.05$ .

Uteri were removed, scraped free of endometrium and disrupted with a Vertis homogeniser. The crude homogenate was filtered through cheesecloth and then centrifuged at 600 and 1,000g for 15 min each. The pellets were discarded after each centrifugation and the resulting supernatant was centrifuged at 30,000g for 15 min. The pellet was resuspended in *N*-2 hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid (HEPES), pH 7.5, 4 mM MgSO<sub>4</sub>, 1 mM dithiothreitol (DTT) and used for [<sup>125</sup>I]IHYP and DHE binding assays. 0.1 ml aliquots of the suspension containing 1.8 mg ml<sup>-1</sup> protein ([<sup>125</sup>I]IHYP assay) or 4.4 mg ml<sup>-1</sup> protein (DHE assay) were added to 0.1 ml HEPES 50 mM, pH 7.5, 4 mM MgSO<sub>4</sub>, 1 mM DTT containing increasing concentrations of radioligand (0.1 to 20 times  $K_d$ ) and 0.02 ml of 1 mM HCl or 10  $\mu$ M (–)-propranolol ([<sup>125</sup>I]IHYP assay) or 10  $\mu$ M phentolamine (DHE assay) in 1 mM HCl. Incubations were at 30 °C for 15 min (DHE) or 60 min ([<sup>125</sup>I]IHYP assay). Binding was terminated in the [<sup>125</sup>I]IHYP assay by adding to the incubation solution 1.5 ml of 0.1 mM (±)-propranolol in 1 mM MgSO<sub>4</sub>, 20 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.5 (wash buffer) and immediately filtering the sample through Gelman A-E glass fibre filters. The filters were washed for 30 s with 30 ml of wash buffer at 37 °C. In the DHE assay the incubation was terminated by adding 5 ml of 5 mM HEPES, pH 7.5 and 2 mM MgSO<sub>4</sub> at 23 °C and immediate filtering through Whatman GF/C filters. The filters were then washed with 15 ml of the same solution at 23 °C. Specific binding is defined as the difference between binding in the absence and presence of 10  $\mu$ M unlabelled antagonist (propranolol in the [<sup>125</sup>I]IHYP assay and phentolamine in the DHE assay). Data were analysed by a nonlinear iterative curve fitting program and normalised to sites per mg DNA<sup>10</sup>. Results are means of individual site number and  $K_d$  determinations from 5 experiments ± s.e.m. Statistical analysis was by Student's unpaired *t* test.

Parallel experiments were carried out in which the filtrate and wash were collected and refiltered after DHE or [<sup>125</sup>I]IHYP binding and the usual filtration. The refiltration was done three times. More than 90% of the specifically bound radioactivity of both ligands was present on the first filter.



**Fig. 1** Scatchard analysis of DHE binding experiments with particulates from oestrogen-treated (●) or oestrogen followed by progesterone-treated (○) rabbits. Data is normalised to binding per mg DNA and points are means of duplicate determinations in five experiments with each treatment.

treated or injected with vehicle instead of progesterone, had numbers of DHE binding sites similar to progesterone-treated animals, indicating that the sites were increased by oestrogen. Quantitation of [ $^{125}$ I] IHYP binding sites demonstrated that  $\beta$ -adrenergic predominance in uteri from progesterone-treated animals could not be explained by an increase in [ $^{125}$ I] IHYP binding sites or by altered affinity of the receptor for [ $^{125}$ I] IHYP. Indeed, there was a small but not statistically significant ( $0.1 > P > 0.05$ ) increase in [ $^{125}$ I]-IHYP binding sites in oestrogen-treated uteri (in which  $\alpha$ -adrenergic response predominates) (Table 1).

DHE binding sites were 10–40 times more numerous than [ $^{125}$ I] IHYP binding sites. The difference in the number of  $\alpha$ - and  $\beta$ -adrenergic binding sites could not be attributed to the filtration procedure used to determine radioactivity bound to the myometrial preparation (Table 1). Quantitation of  $\beta$ -adrenergic binding sites using the lower specific activity radioligand  $^3$ H-dihydroalprenolol also resulted in approximately the same number of binding sites as did [ $^{125}$ I] IHYP. Binding studies of the initial homogenate had high levels of nonspecific binding but demonstrated similar changes in  $\alpha$ -adrenergic binding sites with the different treatments and confirmed the relative differences in numbers of  $\alpha$ - and  $\beta$ -adrenergic sites. In experiments in which we used NaF-stimulated adenylate cyclase to monitor membrane recovery, we found that the differences between the number of  $\alpha$ - and  $\beta$ -adrenergic sites could not be attributed to differences in membrane recovery.

The smaller number of myometrial  $\alpha$ -adrenergic binding sites after progesterone treatment in the rabbit accompanies the conversion of a contractile to an inhibitory response, and this could be a result of the change in the number of binding sites. Whether the change in the ratio of  $\alpha$ - to  $\beta$ -adrenergic receptor sites from 15:1 to 50:1 in the presence of oestrogen is by itself sufficient to change myometrial response to catecholamines from inhibitory to contractile cannot presently be determined. Because oestrogen and progesterone, however, have additional effects on myometrial metabolism and function<sup>17–19</sup>, it is possible that the alteration in contractile response results from the interaction of several events. The adrenergic component of other changes observed during pregnancy or treatment with oral contraceptives including cardiac output, vascular reactivity and metabolism, may also be influenced by such altered response<sup>20–24</sup>. Recent studies in liver and heart have demonstrated alterations in adrenergic receptor numbers in response to glucocorticoids and thyroid hormone respectively<sup>1,25,26</sup>.

It has been postulated that  $\alpha$ - and  $\beta$ -adrenergic receptors are structurally related and may in fact represent alternate configurations of the same active site<sup>7–9</sup>. The changes in numbers of binding sites in oestrogen- and progesterone-treated animals cannot be explained by a conversion of  $\beta$ - to  $\alpha$ -adrenergic receptors. The number of  $\beta$ -binding sites was about 5% of the number of  $\alpha$ -binding sites and was not changed with the addition of progesterone, while  $\alpha$  sites decreased during progesterone treatment by 70%. If the decrease in  $\alpha$ -receptor number was attributable to a conversion of  $\alpha$  to  $\beta$  receptors, up to a 40-fold increase in  $\beta$  receptors would have been anticipated. This direct estimate of  $\alpha$ - and  $\beta$ -adrenergic receptors does not support the hypothesis that the receptors are simply alternate configurations of the same gene product(s), and is more compatible with the concept that they are separate entities.

These studies suggest an important role for the regulation of adrenergic receptors by hormones acting at the level of the cell nucleus, and complement studies which have demonstrated regulation of adrenergic receptors by adrenergic agonists<sup>27–29</sup>.

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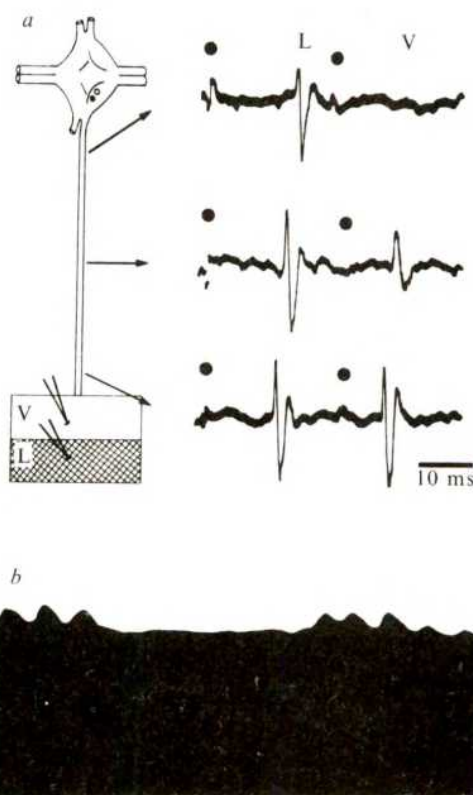
- Wolfe, B. B., Harden, T. K. & Molinoff, P. B. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1343–1347 (1976).
- Rajerson, R., Marchetti, J., Roy, C., Bockaert, J. & Jard, S. *J. biol. Chem.* **249**, 6390–6400 (1974).
- Exton, J. H., Friedmann, N., Wong, E. H., Brineaux, J. P., Parks, C. R. & Corbin, J. D. *J. biol. Chem.* **247**, 3579–3588 (1972).
- Miller, M. D. & Marshall, J. M. *Am. J. Physiol.* **209**, 859–865 (1965).
- Miller, J. W. *Ann. N.Y. Acad. Sci.* **139**, 788–798 (1967).
- Iverson, L. L. *Frontiers in Catecholamine Research*, 3rd International Catecholamine Symposium, University of Strasbourg (eds Usdin, E. & Snyder, S. H.) 403 (Pergamon, New York, 1973).
- Kunos, G. *Br. J. Pharmac. Chemother.* **59**, 177–189 (1977).
- Matheny, J. L. & Ahlquist, R. P. *Arch. Int. Pharm. Therap.* **218**, 4–10 (1975).
- Kunos, G., Yong, M. S. & Nickerson, M. *Nature new Biol.* **241**, 119–120 (1973).
- Giles, K. W. & Myers, A. *Nature* **206**, 93 (1965).
- Williams, L. T., Mullikin, D. & Lefkowitz, R. J. *J. biol. Chem.* **251**, 6915–6923 (1976).
- Roberts, J. M., Insel, P. A., Goldfien, R. D. & Goldfien, A. *Endocrinology* (in the press).
- Maguire, M. E., Wiklund, R. A., Anderson, H. J. & Gilman, A. G. *J. biol. Chem.* **251**, 1227–1231 (1976).
- Brown, E. M., Aurbach, G. D., Hauser, D. & Troxler, F. *J. biol. Chem.* **251**, 1232–1238 (1976).
- Harden, T. K., Wolfe, B. B. & Molinoff, P. B. *Molec. Pharmac.* **12**, 1–15 (1976).
- Insel, P. A., Maguire, M. E., Gilman, A. G., Bourne, H. R., Coffino, P. & Melmon, K. L. *Molec. Pharmac.* **12**, 1062–1069 (1976).
- Rinard, G. A., Chew, C. S. *Life Sci.* **16**, 1507–1512 (1975).
- Roberts, J. S., Barcikowski, B., Wilson, L., Skarnes, R. C. & McCracken, J. A. *J. Steroid Biochemistry* **6**, 1091–1097 (1975).
- Bulbring, E. in *Drug Receptors* (ed. Rang, H. P.) 1–13 (University Park Press, London, 1973).
- Ueland, K., Novy, M. J. & Metcalfe, J. *Am. J. Obstet. Gynec.* **115**, 4–10 (1973).
- Elgee, N. *J. Ann. Int. Med.* **72**, 409–418 (1970).
- Spellacy, W. N., Carlson, K. L., Birk, S. A. & Schade, S. L. *Metabolism* **17**, 496–501 (1968).
- Talledo, O. E., Chesley, L. C. & Zuspan, F. P. *Am. J. Obstet. Gynec.* **100**, 218–221 (1968).
- Lehtovirta, P. *J. Obstet. Gynaec. Br. Commonw.* **81**, 517–525 (1974).
- Ciaraldi, T. & Marinetti, G. V. *Biochem. Biophys. Res. Comm.* **74**, 984–991 (1977).
- Williams, L. T., Lefkowitz, R. J., Watanabe, A. M., Hathaway, D. R. & Besch, H. R. *J. biol. Chem.* **252**, 2787–2789 (1977).
- Strittmatter, W. J., Davis, J. N. & Lefkowitz, R. J. *J. biol. Chem.* **252**, 5478–5482 (1977).
- Mickey, J. V., Tate, R., Mullikin, D. & Lefkowitz, R. J. *Molec. Pharmac.* **12**, 409–419 (1976).
- Shear, M., Insel, P. A., Melmon, K. L. & Coffino, P. *J. biol. Chem.* **251**, 7572–7576 (1976).



## Killing of single neurones by intracellular injection of proteolytic enzymes

To approach problems of sprouting, regeneration, and specificity of neuronal connections, it would be useful if a technique were available for selectively destroying an individual neurone or group of neurones without damaging other cells. One established method for eliminating a class of cells is to use the genetic approach and produce mutants. This, however, is often impractical for animals with long generation times. In invertebrate preparations, surgical removal of the cell body is not sufficient, since neuronal processes can survive and continue to function normally for weeks or months after they have been severed from their cell bodies<sup>1,2</sup>. We have used the central nervous system of the leech to establish a technique for destroying individual neurones one at a time. The leech is a convenient preparation since the nerve cells are large, easily recognisable, and many have been identified and their synaptic connections and peripheral fields are well known<sup>3,4</sup>. We have found that Pronase, a mixture of proteolytic enzymes (Calbiochem Pronase, free of nucleases, B grade), can be injected by pressure into an identified cell. With time, it spreads throughout the neurone's central and peripheral processes and destroys them.

The experiments were made with microelectrodes filled with 0.5% Pronase, 2% dye (fast green) and 50 mM KCl.



**Fig. 1** *a*, Impulses recorded extracellularly from the axons of two sensory cells innervating lateral (L) and ventral skin (V) (ref. 3). One cell (V) had been injected with Pronase 18 h beforehand when its receptive field and that of L were mapped. In the experiment, impulses initiated by touching ventral skin failed to propagate into the ganglion while those from lateral skin continued to do so. The dots above the records indicate the instant at which the skin was indented by the piezo electric crystal. *b*, Photographic silhouette taken of part of a leech in which two motor neurones (in adjacent ganglia) responsible for reflex erection of the skin into ridges were injected with Pronase. The region of skin normally innervated by two cells (six annuli) lost the reflex.

The dye allowed visual monitoring of the injection and the KCl allowed the membrane potential to be recorded. The tips of the electrodes were bevelled to diameters between 1 and 2  $\mu$ m and pressures of 1 to 5 pounds per square inch were applied. Injections usually took less than 5 s and the cell's resting and action potentials were still normal at the time the electrode was removed. Injections were made in isolated ganglia and in whole animal preparations. Isolated ganglia were cultured for periods of days after the injections<sup>5</sup>. Experiments using whole animals were carried out by anaesthetising them with 0.15% chlorobutanol and making a small incision through the ventral skin to expose a single ganglion. With oblique illumination it was possible to recognise individual cells and to confirm their identity by the characteristic shape of the action potentials<sup>3</sup>. The animals survived for many weeks at room temperature after the operations.

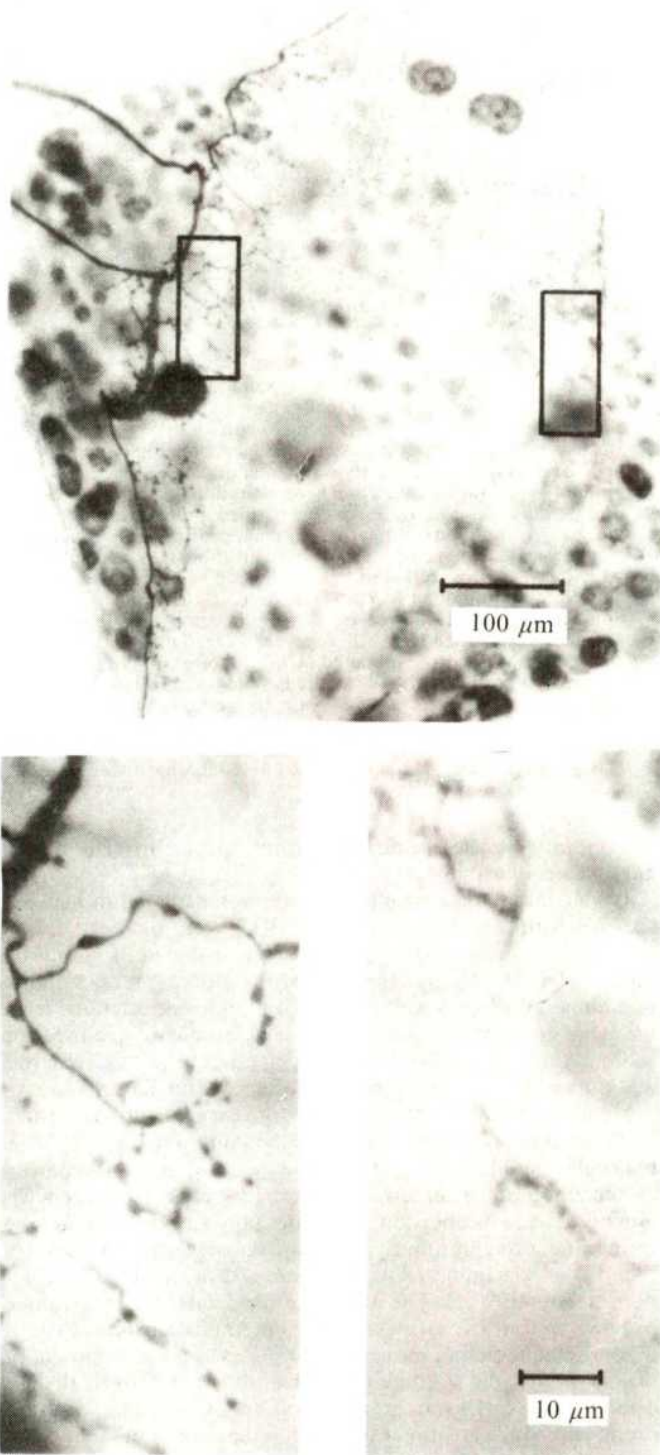
To follow the progress of cell destruction, a ganglion was removed still attached to the segment of skin it innervates. A mechanosensory cell that responds to light touch<sup>3</sup> (touch cell) was injected with Pronase. Its sensory terminals were stimulated by a piezo electric crystal that indented the skin and extracellular recordings of the evoked action potentials were made from the nerve root connecting the skin to the ganglion. As a control, a neighbouring touch cell that innervates an adjacent area of skin was stimulated with a second crystal.

During the first 1 or 2 h after the injection, the cell body progressively lost its resting potential and ability to generate action potentials. Mechanical stimulation, however, of the skin still evoked action potentials travelling toward the ganglion that could be recorded from the roots. Over the next 20 h, conduction of action potentials along the axon failed at greater distances from the cell body (Fig. 1*a*) and by 48 h, the most distal process, millimeters from the site of the injection, was unresponsive. Conduction along the process of the control touch cell (Fig. 1*a*, L) which runs in the same nerve root, was not affected. In similar experiments in which a touch cell was injected in the whole animal, no recovery of function was observed over a 2-week period and the injected cell body could no longer be seen in the ganglion under a dissecting microscope. In control experiments cells that were injected with fast green and KCl alone showed none of these degenerative changes.

Functional evidence that the injected cells were destroyed was provided by injecting annulus erector motor neurones that are part of a reflex arc responsible for erection of the annuli into ridges<sup>4</sup>. Figure 1*b* is a photograph showing a silhouette of part of a leech in which a single motor neurone was injected in each of two neighbouring ganglia in a whole animal. Within 24 h the reflex was lost over the 6 annuli that are innervated exclusively from these neurones<sup>4</sup>. No recovery was apparent over a period of several weeks. Mechanical stimulation of the skin in dissected preparations from these animals continued to evoke normal sensory responses. When the ganglia were inspected, the motor neurones could not be seen but the ganglia appeared normal otherwise.

Anatomical evidence for the spread of Pronase through the cell's fine branches and into its terminals was provided by experiments in which neurones were injected with horseradish peroxidase (HRP) (ref. 6) and then with Pronase. Figure 2 shows an experiment in which two touch cells were injected with HRP. Four hours later, the cell on the right side of the ganglion was injected with Pronase, the cell on the left with a solution of KCl and dye without Pronase. Development of the cells for HRP staining 12 h later showed almost no reaction product in the cell body or central processes of the Pronase-injected cell. The reaction product, however, was dense in the cell's more distal processes in roots and connectives (out of the field) where the Pronase presumably had not yet reached, demonstrating





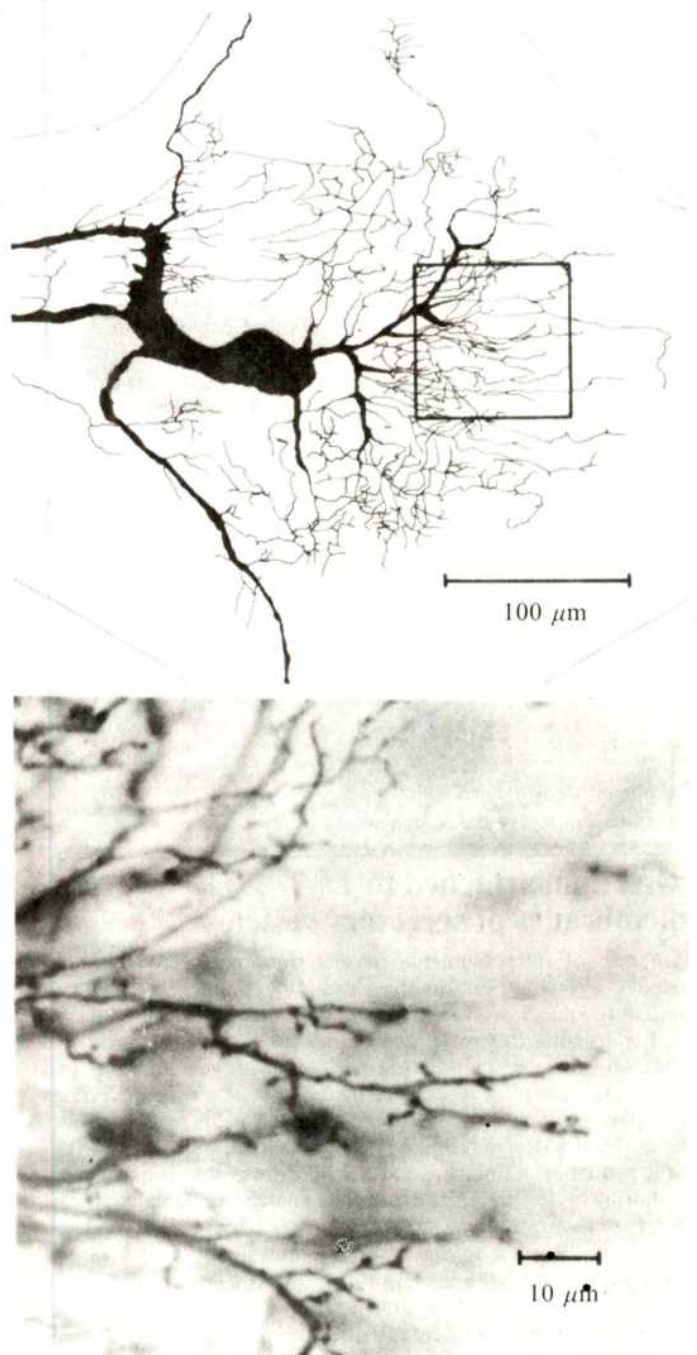
**Fig. 2** Leech ganglion in which one touch cell on each side of the ganglion was injected with HRP. Four hours later, the touch cell on the right was injected with Pronase. After another 15 h, the peroxidase reaction was allowed to develop. The cell injected with Pronase showed only scattered processes within the neuropil but its axons could still be discerned at a distance from the cell body in roots and connectives, indicating that the HRP injection had been successful.

that the HRP injection had been successful. Processes of the control cell appeared normal.

It is important to establish that the direct effects of Pronase are confined to the injected cell. We have not been able to devise a quantitative assay for direct damage to other neurones, but a number of tests have failed to reveal any characteristic morphological or physiological changes in the properties of other cells. Injecting Pronase extra-

cellularly under the connective tissue capsule around the ganglion had no apparent effect on the electrical properties of the cells. Cells that are synaptically coupled to or in close proximity to an injected cell seemed to be anatomically and physiologically normal. For example, one of the two Retzius cells was injected with Pronase and two weeks later, the ganglion was removed from the animal and the remaining Retzius cell was injected with HRP (Fig. 3). Although the two Retzius cells are electrically coupled and form synapses in the same region of the neuropil, the fine

**Fig. 3** Camera lucida drawing of a Retzius cell injected with HRP and a photograph of some of its fine processes in the neuropile. Two weeks earlier, the other Retzius cell in the ganglion had been injected with Pronase. The Pronase-injected Retzius cell had disappeared by this time but the arborisation of the cell injected with peroxidase (which had been coupled to the Pronase-injected cell) still appears normal. The stippled area represents the cell body.





processes of the remaining cell appeared to be undamaged and its physiological properties were unchanged. Results from this cell and other experiments showed that the synaptic potentials, action potentials, and input impedances of non-injected cells remained normal. No obvious changes were found even when a large number of other cells in the ganglion (up to a dozen) were injected with Pronase. The fine structure of non-injected cells also seemed normal. A particularly favourable cell to investigate by electron microscopy is the S neurone<sup>7</sup>. This cell sends a large axon through the middle connective (Faivre's nerve) that can readily be identified by its size and position. After an S cell was injected with Pronase, its degenerating axon could be seen in Faivre's nerve, but the surrounding axons and glial processes appeared normal (also K. Muller, personal communication).

Although these experiments do not rule out more subtle forms of damage to fine processes or to cell surfaces caused by Pronase leaking out of the injected cell, we conclude that injection of Pronase destroys all the processes of a single neurone without obvious damage to other cells. This technique may be particularly useful in simple invertebrate preparations, like the leech, in which one can now study the effects of removing one cell at a time instead of making less selective lesions that involve hundreds or even thousands of axons. For example, if a cell is killed, will sprouting occur in cells that are normally pre- or post-synaptic to it? Will processes grow to form new synapses replacing terminals removed by the Pronase injection? Even without obvious morphological changes, the efficacy of synaptic transmission between two cells might be altered by removal of a third neurone supplying input to the synapse. The technique may also be useful in determining functional roles of specific neurones and for analysing their connections to other cells.

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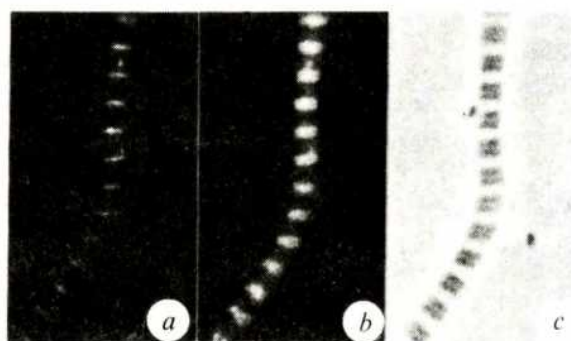
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1. Hoy, R. R., Bittner, G. D. & Kennedy, D. *Science* **156**, 251–252 (1967).
2. Van Essen, D. C. & Jansen, J. K. S. *J. comp. Neurol.* **171**, 433–454 (1977).
3. Nicholls, J. G. & Baylor, D. A. *J. Neurophysiol.* **31**, 740–756 (1968).
4. Stuart, A. E. *J. Physiol., Lond.* **209**, 627–646 (1970).
5. Miyazaki, S. & Nicholls, J. G. *Proc. R. Soc. Lond. B* **194**, 295–311 (1976).
6. Muller, K. J. & McMahan, U. J. *Proc. R. Soc. Lond. B* **194**, 481–499 (1976).
7. Carbonetto, S. & Muller, K. J. *Nature* **267**, 450–452 (1977).

## $\alpha$ -Actinin attached to membranes of secretory vesicles

THE role of microfilaments in secretion has been much debated<sup>1–4</sup>. If secretion involves a sliding filament mechanism similar to muscle contraction<sup>5</sup>, microfilaments must attach at least transiently to vesicle membranes. Indeed, there is evidence that contractile proteins are associated with secretory vesicles (chromaffin granules) of adrenal medulla<sup>6,7</sup>. Moreover, the outer (cytoplasmic) surface of secretory vesicles must provide 'anchoring molecules' for microfilaments. In skeletal muscle, the structural protein  $\alpha$ -actinin located in the Z-line<sup>8</sup> serves as such an anchoring molecule. An  $\alpha$ -actinin-like protein was isolated from a variety of non-muscle cells<sup>9,10</sup> and there is evidence that the same protein is attached to the inner (cytoplasmic) surface of the plasma membrane of non-muscle cells in association with microfilaments<sup>11–14</sup>. Using a monospecific antibody to porcine  $\alpha$ -actinin (Fig. 1) we have demonstrated the presence of this protein



**Fig. 1** Rat myofibril stained by means of indirect immunofluorescence. Antibody was raised in rabbits against purified native porcine skeletal muscle  $\alpha$ -actinin<sup>15</sup> precipitated with alum<sup>13</sup>. A crude IgG fraction was passed over an  $\alpha$ -actinin Sepharose affinity column<sup>16</sup> and the monospecific antibody was eluted at pH 2.7 (ref. 17). This antibody preparation gave a single precipitation line when tested in double diffusion against crude or purified preparations of the antigen. An antibody-Sepharose column retained only  $\alpha$ -actinin from a muscle homogenate. The isolated myofibril (c) was incubated with this antibody followed by a, rhodamine-conjugated sheep antiserum against rabbit IgG (ref. 18); then b, incubated with human anti-actinin serum<sup>19</sup> followed by fluorescein-conjugated goat antiserum against human IgG (Miles Servac, Switzerland). The myofibril was then washed and mounted in 90% glycerol, 10% phosphate-buffered saline. The width of the rhodamine-positive bands and the distance between them (2.35  $\mu$ m) (a) as well as the comparison between a and b indicate that the antibody against  $\alpha$ -actinin is fixed exclusively to the Z-lines. Preincubation of the anti- $\alpha$ -actinin antibody with purified  $\alpha$ -actinin abolished only staining of the Z-lines.

in secretory vesicle membranes from chromaffin cells of the adrenal medulla and from platelets.

Bovine adrenal chromaffin granules were isolated in buffered sucrose with or without 0.15 M KCl (ref. 6). 5-Hydroxytryptamine-storing (5-HT) vesicles were isolated from rabbit blood platelets by gradient centrifugation<sup>20</sup>; mitochondria and alpha granules<sup>21</sup> were recovered in different fractions from the same gradient. Aliquots of the same gradient were used for sodium dodecyl sulphate (SDS) gel electrophoresis, electron microscopy and indirect immunofluorescence on frozen sections.

Membranes which were prepared from isolated chromaffin and 5-HT granules by hypotonic lysis contain polypeptides with molecular weights of  $\alpha$ -actinin and actin (Fig. 2). Preparing chromaffin granules in low ionic strength buffers (without KCl) diminishes the proportion of those polypeptides among the membrane proteins (data not shown), suggesting a selective removal of  $\alpha$ -actinin at low ionic strength with the actin component bound to it<sup>6</sup>. The actin-like band in the 5-HT granule profile is also clearly distinguishable but not as conspicuous as in the chromaffin granule pattern. The profiles of platelet mitochondria (Fig. 2c) and  $\alpha$ -granule polypeptides (not shown) do not demonstrate any band at the position of  $\alpha$ -actinin. A very prominent band can also be detected in the platelet mitochondria pattern at the position of actin. The proportion of this actin-like component among the total proteins varied considerably (by a factor of 2) with different preparations.

Indirect immunofluorescence was carried out on 4- $\mu$ m frozen sections of pelleted chromaffin granules, 5-HT granules, platelet mitochondria and  $\alpha$ -granules.  $\alpha$ -Actinin antibodies were fixed only to chromaffin granules from adrenal medulla and platelet 5-HT granules (Fig. 3c), whereas  $\alpha$ -granules and platelet mitochondria (Fig. 3d) showed no fluorescence. All fractions were confirmed by electron microscopy to be pure homogeneous preparations. Furthermore, chromaffin granule membranes isolated in high ionic strength buffer (0.15 M KCl) and solubilised in 1% SDS showed a single line of precipitin reaction in a double-diffusion test against  $\alpha$ -actinin antibody.

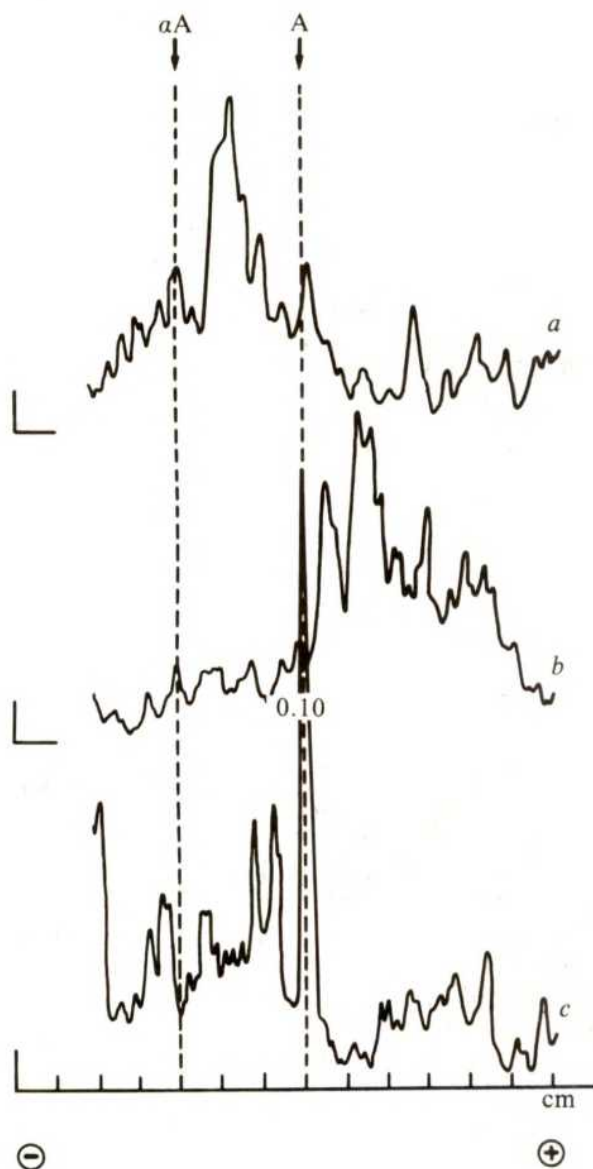
Parallel immunofluorescent staining with antibodies against actin<sup>19</sup> was positive on several fractions from rabbit platelets, including  $\alpha$ -granules and mitochondria. This result, together with the finding of a variable amount of an actin-like protein



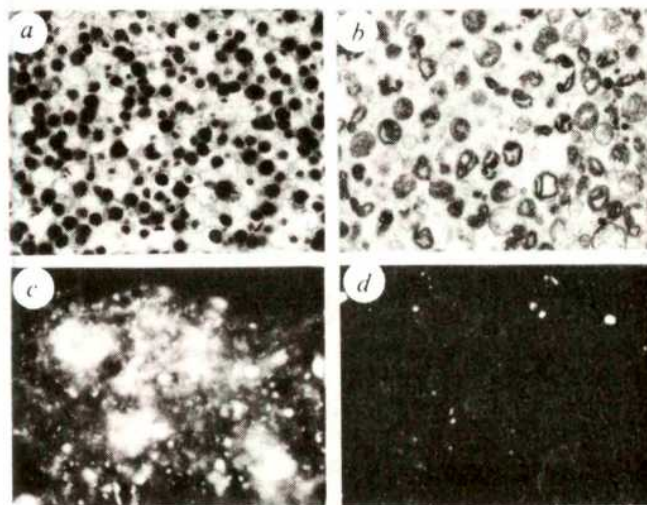
among the mitochondrial polypeptides (Fig. 2) may reflect a contamination of platelet mitochondria with actin from the platelet cytoplasm, although by morphological criteria, these preparations were homogeneously pure (Fig. 3). In the adrenal medulla preparations immunofluorescent staining of different fractions with anti-actin was positive only on the chromaffin granules.

These data indicate that  $\alpha$ -actinin is a constituent of the membrane of monoamine storage vesicles, but not of cellular organelles in general, and they add more weight to the hypothesis that  $\alpha$ -actinin in plasma membranes and secretory vesicle membranes serves as an anchorage molecule for microfilaments.

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**Fig. 2** Densitometer scans of membrane polypeptides stained after electrophoresis on SDS-polyacrylamide slab gels. Bovine adrenal chromaffin granules were prepared in sucrose buffered with 0.02 M 2-(*N*-morpholino) ethane-sulphonic acid (MES), with or without 0.15 M KCl (ref. 6). 5-HT granules and mitochondria were isolated from rabbit blood platelets using a continuous Urografin (Schering, West Germany) gradient as previously described<sup>20</sup>. Membranes from chromaffin and 5-HT granules were prepared by lysing and washing the vesicles in buffer without sucrose. They were then solubilised by boiling them in 1% SDS for 2 min, subsequently subjected to gel electrophoresis<sup>22</sup>, and stained with Coomassie Brilliant Blue. *a*, Chromaffin granule membranes from vesicles isolated in 0.15 M KCl; *b*, 5-HT granule membranes; *c*, platelet mitochondria proteins. The arrows indicate the positions of  $\alpha$ -actinin ( $\alpha$ -A) and actin (A) standards applied to the same gel.



**Fig. 3** Electron micrographs (*a, b*) and immunofluorescence staining (*c, d*) of platelet 5-HT granules (*a, c*) and platelet mitochondria (*b, d*). Aliquots of the same gradients were used. For electron microscopy, the material was fixed in 3% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated and embedded in Epon 812. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a Philips 300 electron microscope. Indirect immunofluorescence was performed on 4- $\mu$ m sections obtained by cutting the frozen material on a cryostat. The incubation procedure with the first (monospecific anti- $\alpha$ -actinin) and the second (FITC-GAR) antibody is described in Fig. 1. Photographs were taken on a Zeiss UV photomicroscope with epi-illumination using Anscochrome colour slide film 500 daylight (Gaf Corporation, New York) or HP4 Ilford film. Magnification: *a, b*,  $\times 10,000$ ; *c, d*,  $\times 400$ .

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1. Allison, A. C. in *Locomotion of Tissue Cells*, Ciba Fdn Symp. **14** (ed. Porter R. & Fitzsimons, D. W.) 109-143 (Associated Scientific, Amsterdam, 1973).
2. Cooke, P. & Poinner, A. M. *Cytobiology* **13**, 442-450 (1976).
3. Orzi, L., Gabbay, K. H. & Malaisse, W. J. *Science* **175**, 1128-1130 (1972).
4. Orr, T. S. C., Hall, D. & Allison, A. C. *Nature* **236**, 350-351 (1972).
5. Huxley, H. E. *Science* **164**, 1356-1366 (1969).
6. Burridge, K. & Phillips, J. H. *Nature* **254**, 526-529 (1975).
7. Gabbiani, G., Da Prada, M., Richards, G. & Pletscher, A. *Proc. Soc. exp. Biol. Med.* **152**, 135-138 (1976).
8. Masaki, T., Endo, M. & Ebashi, S. *J. Biochem.* **62**, 630-632 (1967).
9. Schollmeyer, J. V. *et al.* *J. Cell Biol.* **63**, 304a (1974).
10. Lazarides, E. & Burridge, K. *Cell* **6**, 289-298 (1975).
11. Mosseker, M. S. in *Cold Spring Harbor Conferences on Cell Proliferation*, **3B**, 631-650 (Cold Spring Harbor Labs, 1976).
12. Schollmeyer, J. L., Furcht, L. T., Goll, D. E., Robson, R. M. & Stromer, M. H. in *Cold Spring Harbor Conferences on Cell Proliferation*, **3A**, 361-387 (Cold Spring Harbor Labs., 1976).
13. Lazarides, E. *J. Cell Biol.* **68**, 202-219 (1976).
14. Franke, W. W. *et al.* *J. microsc. Biol. Cell* (in the press).
15. Goll, D. E., Suzuki, A., Temple, J. & Holmes, G. R. *J. molec. Biol.* **67**, 469-488 (1972).
16. Cuatrecasas, P., Wilcheck, M. & Anderson, C. B. *Proc. natn. Acad. Sci. U.S.A.* **61**, 636-643 (1968).
17. Fuller, G. M., Brinkley, B. R. & Boughter, J. M. *Science* **187**, 948-950 (1975).
18. Lamelin, J. P., Lisowska-Bernstein, B., Matter, A., Ryser, J. E. & Vassalli, P. *J. exp. Med.* **136**, 984-1007 (1972).
19. Chaponnier, C., Kohler, L. & Gabbiani, G. *Clin. exp. Immunol.* **27**, 278-284 (1977).
20. Da Prada, M., Berlepsch, K. & Pletscher, A. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol.* **275**, 315-322 (1972).
21. Mustard, J. F. & Packham, M. A. *Pharmac. Rev.* **22**, 97-187 (1970).
22. Laemmli, U. K. *Nature* **227**, 680-685 (1970).



## Direct association of Balbiani ring 75S RNA with membranes of the endoplasmic reticulum

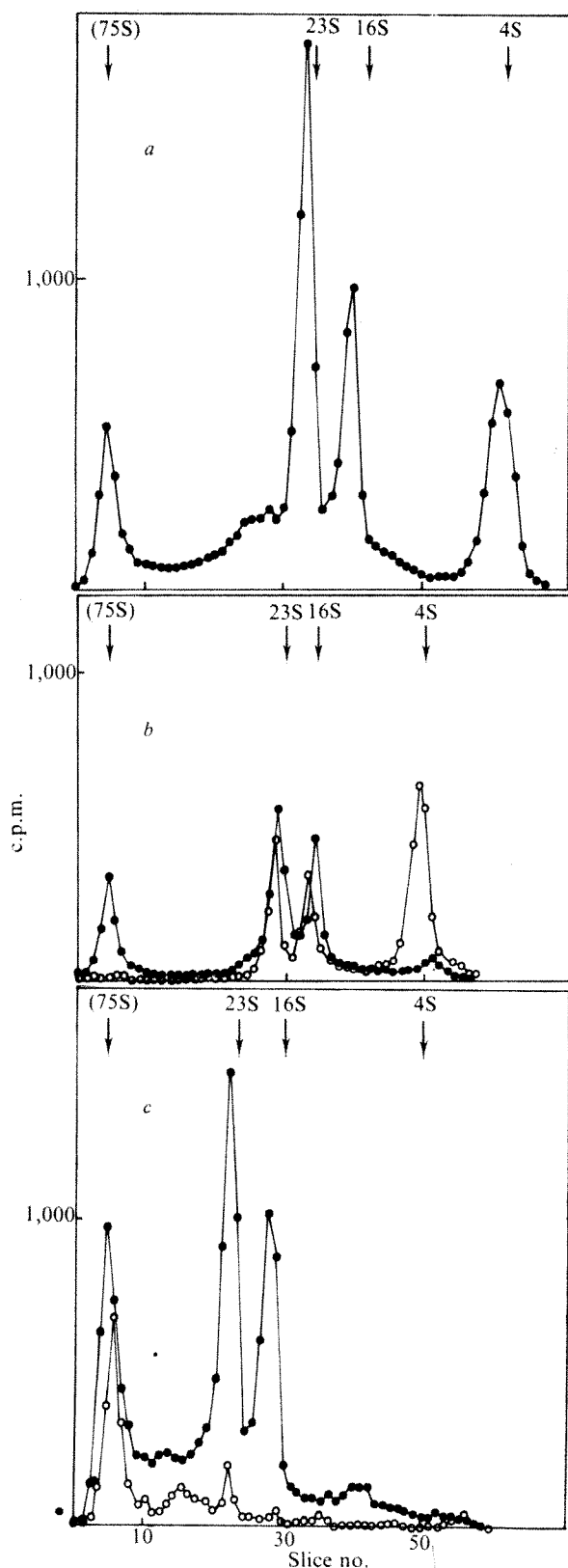
PUFFS in polytene chromosomes probably represent active genes (see, for example, ref. 1). Cytogenetic investigations with large tissue-specific puffs, the Balbiani rings (BR) in *Chironomus* salivary gland cells, revealed a correlation

between the BR and salivary export proteins<sup>2,3</sup>. Biochemical analysis of the BR showed that in *Chironomus tentans* RNA of a defined size is synthesised and transported to the cytoplasm<sup>4</sup>. This RNA, which sediments as 75S RNA, has properties of a messenger RNA, that it, it contains poly A<sup>5</sup> and is located in polysomes<sup>6</sup>. If the BR-RNA directs synthesis of export proteins it would be expected to associate with the membranes of the endoplasmic reticulum (ER). Here we show that such an association exists and that it is independent of ribosomes and polysomal structures.

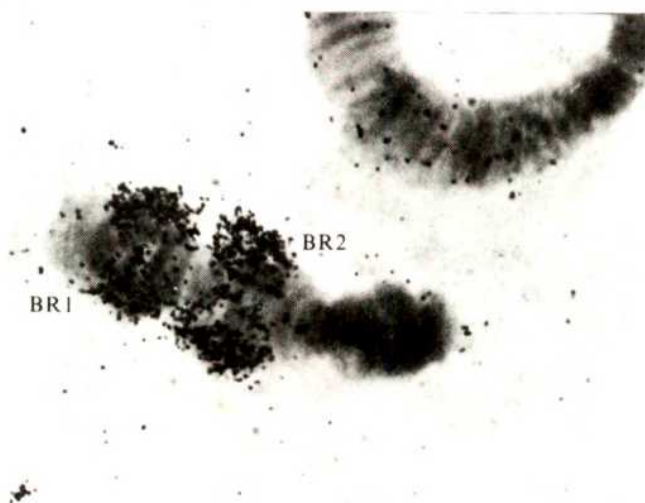
Labelled salivary gland RNA was obtained by micro-injection of tritiated RNA precursors 24 h before sacrifice of the animal. The labelling procedure has the character of a pulse which has declined 1–2 d after injection<sup>7</sup>. At 18 h the nucleus contains 2–4% of total labelled RNA and at 6 d it contained 1%. Thus from 18 h and onwards the risk of contamination of the cytoplasm with labelled nuclear RNA is very low or insignificant. Also, since the half-life of 75S RNA is about 20 h (Edström, J.-E., Lindgren, S., Lönn, U. and Rydlander, L., in preparation) analysis of these animals sacrificed 24 h after injection should give representative samples of the cytoplasm.

The distribution of total labelled RNA in agarose gel electrophoresis is shown in Fig. 1a: over 95% of the RNA visualised in this way is cytoplasmic RNA. The major constituents are the two ribosomal RNA species (28S and 18S RNA) and 4S RNA. There is also a prominent mono-disperse 75S RNA peak of Balbiani ring origin.

The salivary glands were homogenised in low salt buffer containing Mg<sup>2+</sup> and the microsomal membranes pelleted by differential centrifugation<sup>8</sup>. It is difficult to keep 75S RNA intact during aqueous fractionation of the salivary gland cells. But, if sucrose is omitted and the centrifugation times scaled down, as in the procedure described here, there is very little, or no degradation of the RNA. This is evident if one compares the labelled RNA from glands homogenised directly in detergent (Fig. 1a) with those fractionated in low salt buffer before detergent treatment (Fig. 1b). In



**Fig. 1** *a*, Larvae of *Chironomus tentans* were injected with 25  $\mu$ Ci tritiated uridine (50 Ci mmol<sup>-1</sup>; Amersham Radiochemical Centre) 24 h before killing. The excised salivary glands from two animals were homogenised in 0.5 ml 1% sodium dodecyl sulphate (SDS) in 0.02M Tris-HCl pH 7.4 for 5 min at 25°C. The RNA was ethanol-precipitated and separated in 1.5% agarose slab gels<sup>12</sup>. *E. coli* RNA was used as carrier (23S, 16S and 4S RNA). *b*, To determine the amount of membrane-bound 75S RNA a procedure that separates membranes from cytosol was used<sup>8</sup>. The excised salivary glands were allowed to swell for a couple of minutes in 0.5 ml cold reticulocyte standard buffer (1.5 mM MgCl<sub>2</sub>, 10 mM KCl, 10 mM Tris-HCl pH 7.4) and homogenised in a Potter-Elvehjem glass homogeniser. To remove polytene chromosomes and so on the homogenate was centrifuged for 2 min at 750g<sub>max</sub> and to pellet the membranes the supernate thus obtained was centrifuged for another 5 min at 27,000g<sub>max</sub>. To release RNA the 27,000g<sub>max</sub> supernate was made 1% with respect to SDS, whereas the pellet was extracted by addition of 0.5 ml 1% SDS in 0.02 M Tris-HCl pH 7.4. The samples were ethanol-precipitated after 5 min at 25°C. Electrophoresis was performed in 1.5% agarose slab gels<sup>12</sup>. ●, 27,000g<sub>max</sub> pellet, ○, 27,000g<sub>max</sub> supernate. Earlier control experiments showed that 87% of labelled phospholipids appeared in the 27,000g<sub>max</sub> pellet<sup>8</sup>. Also, over 80% of total labelled heavy ribosomal subunits appeared in the pellet at steady-state distribution (6 d after precursor injection)<sup>8</sup>, whereas after 24 h only about 50% appeared in the 27,000g<sub>max</sub> pellet. *c*, To release ribosomes from the microsomal membranes a homogenate prepared as in *b* was divided into two equal aliquots, one of which served as a control. Cold buffer was added to the control aliquot to restore the original volume and immediately centrifuged to pellet the microsomal membranes. To the other aliquot an equal volume of high salt buffer was added (2 M KCl, 50 mM Tris-HCl pH 7.4, 2 mM puromycin, 10 mM EDTA<sup>9</sup>). After incubation for 30 min at 4°C the membranes were pelleted. The pellets were extracted with 1% SDS and the RNA separated in 1.5% agarose slab gels. ○, Stripped membranes; ●, control membranes.



**Fig. 2** Autoradiograph of chromosome IV of *Chironomus tentans* salivary gland cells after *in situ* hybridisation with RNA from stripped membranes. The RNA sample, obtained from membranes stripped of ribosomes as in Fig. 1c and passed through a Sephadex G-25 column, was dissolved in 15  $\mu$ l 2 $\times$ SSC (SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) and added to salivary gland squashes. The squashes were previously denatured in 90% formamide in 0.1 $\times$ SSC at 62–64 °C for 2 h. The hybridisation reaction was carried out at 62–64 °C for 4 h. The subsequent treatments (RNase-treatment, washing in 2 $\times$ SSC and dehydration) was done according to ref. 13. Autoradiography was performed with Kodak AR 10 stripping film; exposure time was 12 weeks. The length of chromosome IV is about 80  $\mu$ m.

control experiments, heating the RNA sample in 8 M urea caused no significant degradation of 75S RNA<sup>8</sup>.

The distribution of labelled RNA between the membrane pellet and its corresponding supernatant is shown in Fig. 1b. The membrane pellet contained 75S RNA and two ribosomal RNA species (28S and 18S RNA). The supernatant contains in addition to these RNA species also 4S RNA. The major part (more than 90%) of 75S RNA appeared in the membrane-fraction.

Further experiments were performed to establish whether it is possible to release ribosomes from the microsomal membranes still keeping the 75S RNA associated to the membranes. To strip the membranes free of ribosomes the membranes were treated *in vitro* with high ionic strength KCl buffers with EDTA and puromycin for 30 min at +4 °C (ref. 9). Gel electrophoresis showed that there was a nearly complete removal of 18S RNA from the membranes. The 28S RNA was removed to 80–90% but over 50% of the 75S RNA remained attached to the membranes (Fig. 1c). Control experiments showed that less than 10% of the 75S RNA appeared in the membrane pellet when it was treated with detergent (0.5% DOC and 0.5% Tween 80) and recentrifuged.

*In situ* hybridisation with labelled RNA from membranes treated with KCl-EDTA-puromycin showed grains over BR one and two (Fig. 2). No other loci, including the nucleolar region, hybridised significantly. In controls with total RNA, also the nucleolar region hybridised significantly. Thus RNA with sequences complementary to the DNA of BR one and two is located in the membrane pellet after the complete release of 18S RNA and the release of the major part of 28S RNA. This indicates that there is in all probability a 75S RNA-membrane interaction which is independent of ribosomes and polysomal structures.

Similar findings have been obtained in fibroblast<sup>10</sup>, liver<sup>9</sup> and HeLa cells<sup>11</sup> where the attachment of mRNA to microsomal membranes was shown to exist via the 3'-end containing the poly A tail. 75S RNA also contains poly A (ref. 5) but it is not known whether it is responsible for the attachment.

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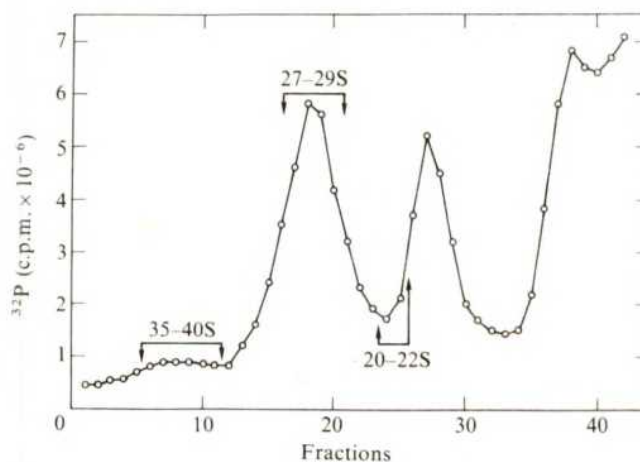
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1. *Result and Problems in Cell Differentiation 4, Developmental Studies on Giant Chromosomes* (Springer, Berlin, 1972).
2. Beerman, W. *Chromosoma* **12**, 1–25 (1961).
3. Grossbach, U. *Chromosoma* **28**, 136–187 (1969).
4. Daneholt, B. & Hosick, H. *Proc. natn. Acad. Sci. U.S.A.* **70**, 442–446 (1973).
5. Edström, J.-E. & Tanguay, R. *J. molec. Biol.* **84**, 569–583 (1974).
6. Daneholt, B., Andersson, K. & Fagerlind, M. *J. Cell Biol.* **73**, 149–160 (1977).
7. Lönn, U. & Edström, J.-E. *J. Cell Biol.* **73**, 696–704 (1977).
8. Lönn, U. *Med. Biol.* (in the press).
9. Cardelli, I., Long, B. & Pitot, H. *J. Cell Biol.* **70**, 47–58 (1976).
10. Lande, M., Adesnik, M., Sumida, M., Tashiro, Y. & Sabatini, D. *J. Cell Biol.* **65**, 513–528 (1975).
11. Milearek, C. & Penman, S. *J. molec. Biol.* **89**, 327 (1974).
12. Edström, J.-E. & Lönn, U. *J. Cell Biol.* **70**, 562–572 (1976).
13. Lambert, B., Wieslander, L., Daneholt, B., Egyházi, E. & Ringborg, U. *J. Cell Biol.* **53**, 407–418 (1972).

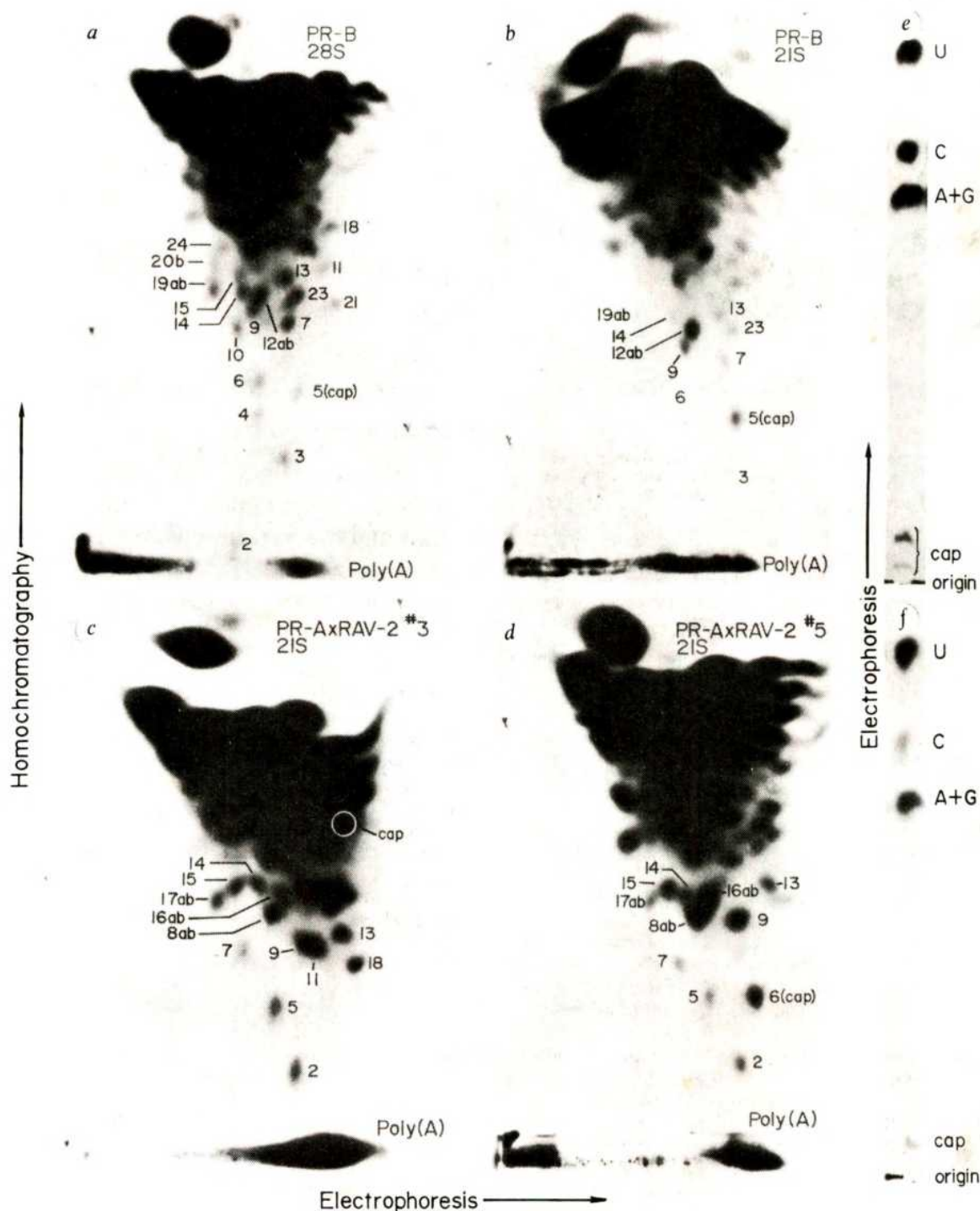
## Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3' half and the 5' terminus of virion RNA

THE genome of Rous sarcoma virus (RSV) is a 30–40S RNA of 10,000 nucleotides coding for four known genes<sup>1</sup> which map in the order 5'-gag, pol, env, src, poly(A)-3' (refs 2, 3). This 30–40S RNA serves as mRNA for a gag precursor protein and perhaps a gag-pol precursor-polypeptide when translated *in vitro*<sup>4,5</sup>. The env gene, however, may be translated from a smaller intracellular messenger. Poly(A)-containing 20–24S cellular RNA from infected cells can, upon microinjection, complement an env-defective virus<sup>6</sup> and may also direct *in vitro* synthesis of a protein that is serologically related to the env gene product<sup>7</sup>. Moreover, hybridisation with DNA complementary (cDNA) to env and src sequences of virion RNA detects discrete classes of subgenomic poly(A)-containing RNAs present in infected cells as steady state species, while gag and pol-specific cDNAs hybridise only to full sized RNA<sup>8,9</sup>.



**Fig. 1** Sucrose gradient sedimentation of cellular RNA from RSV-infected cells. PR-RSV-B-infected cells<sup>2,3,12,13</sup> labelled with 75 mCi (<sup>32</sup>PO<sub>4</sub>)<sup>3-</sup> for 3 h were trypsinised, suspended in 0.1 M NaCl, 0.01 M Tris (7.4), 1 mM EDTA, extracted twice with phenol, chloroform, Na<sub>2</sub>SO<sub>4</sub>, mercaptoethanol, at about 60:38:1:1, and ethanol-precipitated. Nucleic acids were heated to 100 °C in 0.01 M NaCl, 0.01 M Tris (7.4), 1 mM EDTA with 0.1% Na<sub>2</sub>SO<sub>4</sub> and layered onto a gradient of 10–25% sucrose containing the same buffer. Centrifugation was for 6.5 h at 40,000 r.p.m. and 20 °C in an SW41 rotor. Radioactivity was Cerenkov counted and the RNA of the three pools indicated was analysed as described in Fig. 2.





**Fig. 2** Two-dimensional fingerprint analysis of poly(A)-tagged, virus-specific, intracellular RNA and analyses of their 5' terminal, capped-oligonucleotides. Three different size classes of poly(A)-tagged RNA species, isolated from about  $5 \times 10^7$  RSV-cells, as for Fig. 1, were selected by chromatography on oligo(dT)-cellulose as described<sup>2,3,13,12,15</sup>. The RNA of each pool, about  $6-10 \times 10^6$  c.p.m., was mixed with 0.7  $\mu$ g viral poly(dC)-cDNA and heated to 100 °C in 0.01 M NaCl, 0.01 M Tris HCl, pH 7.4, 1 mM EDTA and lyophilised. The mixture was then incubated in 25-50  $\mu$ l 70% formamide, 0.3 M NaCl, 0.03 M Na citrate, 1.5 mM Na phosphate, pH 7.0, at 40 °C for 6-10 h. Subsequently the solution was diluted with 1 ml 0.15 M NaCl, 0.015 M Na citrate and heated at 60 °C for 10 min. RNA-DNA hybrids were selected by chromatography on a 1 ml-column of oligo(dG)-cellulose (Collaborative Research, Waltham, Mass.) by a modification of procedures described previously<sup>20,21</sup>. The conditions were as described for chromatography on oligo(dT)-cellulose, except that the column was washed with 15  $\mu$ g poly(rA) in 1 ml application buffer (0.5 M LiCl, 0.01 M Tris HCl, pH 7.4, 0.05% Na dod SO<sub>4</sub>) and rinsed with 0.5 ml of the same buffer lacking poly (rA) prior to chromatography of the hybrids. The eluted hybrids were made 0.5 M LiCl and rechromatographed. The hybrids were melted by heating to 100 °C at low ionic strength, ethanol-precipitated and the hybridisation-selection process repeated except that only 0.1-0.2  $\mu$ g poly(dC)-cDNA was added and that the hybrids were selected only once on the oligo(dG)-cellulose column. Recovery of poly(A)-tagged RNA as hybrids was between 0.05 and 4% for distinct pools (see text). Eluted hybrids were melted as above and ethanol-precipitated for T<sub>1</sub> digestion and two-dimensional fingerprinting<sup>12,13,15</sup>. PR-B cDNA was used in all experiments. It was synthesised as described<sup>22</sup> and prepared by exclusion chromatography on a Biogel P100 (12  $\times$  0.7 cm, BioRad, Richmond, CA) column in 0.01 M Tris, pH 7.4, 1 mM EDTA and 0.05% Na dod SO<sub>4</sub>. About 6  $\mu$ g cDNA was polycytidylated with 2 units of deoxynucleotidyl transferase (PL-Biochemicals) in 60  $\mu$ l 0.1 M KH<sub>2</sub>PO<sub>4</sub>, 0.05M cacodylic acid, pH 7.0, 1 mM mercaptoethanol, 2 mM CoCl<sub>2</sub>, 3 mM dCTP for 12 h at 40 °C. Poly(dC)-cDNA was purified by chromatography on Biogel P100 as above. Excluded cDNA was then selected on oligo(dG)-cellulose as described above omitting poly(rA). Recovery of poly(dC)-cDNA was 65%. Panels A-D are autoradiographs of T<sub>1</sub>-resistant oligonucleotides of virus-specific RNAs resolved by two-dimensional electrophoresis-homochromatography<sup>2,3,12,13</sup>, termed fingerprinting. (a) Finger-



Neither the structure nor the mechanism of generation of these subgenomic RNA species has been elucidated. They may be produced by partial transcription of the DNA provirus or by functionally specific cleavage of genome-sized RNA. They may also include species generated by structurally specific degradation of viral RNAs. The structure of such RNAs would be that of a contiguous segment of virion RNA with at least one terminus derived from an internal location on the virion RNA. Alternatively, subgenomic viral RNAs could be generated by internal deletions of virion RNA, a mechanism analogous to that which has been discovered to govern the mRNA synthesis of adenovirus, a DNA-containing virus. Some adenovirus mRNAs were found to have a 100–200 nucleotide sequence covalently attached to the 5' end of the coding sequence, but transcribed from a remote segment of the DNA template<sup>10,11</sup>. This observation suggests that covalent linkage of transcripts from non-adjacent DNA templates, termed splicing, may be characteristic of eukaryotic cellular and viral mRNAs.

We have isolated and characterised the poly(A)-tagged RSV-specific RNAs synthesised during a 3-h <sup>32</sup>P labelling period in infected cells, specifically addressing the questions of sequence arrangement and derivation of their terminal sequences. These analyses were done using the large, unique RNase T<sub>1</sub>-resistant oligonucleotides of virion RNA as diagnostic of distinct viral RNA segments<sup>12,13</sup> for two reasons: (i) Since their order relative to the poly(A)-terminus of each viral RNA studied here is already known<sup>2,3,12</sup>, their detection would directly identify those virion RNA segments from which a subgenomic RNA species was derived. (ii) Moreover identification of the 5'-terminal 7mGpppGmC(cap)-oligonucleotide<sup>14,15</sup> of a subgenomic viral RNA species would unambiguously define its 5'-terminus. This cannot be done directly by hybridisation with cDNAs complementary to the termini of virion RNA, because such cDNA detects presumably redundant sequences on both ends of virion RNA in addition to internal sequences<sup>16,17,18</sup> and because positive hybridisation does not indicate where on the RNA a given sequence is located.

Viral-specific poly(A)-containing RNA was isolated from Prague RSV-B (PR-B)-infected cells by the following procedure: RSV-transformed fibroblasts were incubated with media containing [<sup>32</sup>PO<sub>4</sub>]<sup>3-</sup> for 3 h. Nucleic acids, phenol-extracted from these cells, were fractionated by sucrose gradient sedimentation (Fig. 1). Three size classes marked in Fig. 1 were pooled and poly(A)-containing RNA species were selected by chromatography on oligo(dT)-cellulose. The recoveries were 16% for the 35–40S pool, 4% for the 27–29S pool and 20% for the 20–22S pool. Virus-specific RNA was isolated by twice repeated hybridisation to poly(dC)-tagged PR-B cDNA with selection on oligo(dG)-cellulose (see Fig. 2). The recovery of [<sup>32</sup>P]RNA from each original pool was approximately 4% (35–40S), 0.1% (27–29S) and 0.3% (20–22S). The percent virus-specific [<sup>32</sup>P]RNA in the 27–29S and 20–22S species prepared by hybridisation to viral cDNA was 60–80% as determined by resistance to digestion with

with RNase T<sub>1</sub> and analysed by two-dimensional fingerprinting<sup>2,3,12,13</sup>. Each large T<sub>1</sub>-resistant oligonucleotide was identified by its relative chromatographic location and by analysis of its RNase A-resistant fragments (not shown) and numbered as its defined counterpart in virion RNA<sup>2,3,13</sup>. In every case the 35–40S pool was identical to virion RNA (not shown). The T<sub>1</sub>-oligonucleotides present, at approximately equimolar ratios, in the 27–29S intracellular PR-B RNA (Fig. 2a) are those underlined and oligonucleotides present in the 20–22S RNA (Fig. 2b) are those shown in bold face type on the complete oligonucleotide map of PR-B RNA: nucleotide (ref. 24) of the 20–22S PR-B RNA gave the following near equimolar amounts expressed as c.p.m. per phosphate: 5(cap): 3.5, 13: 2.5, 23: 3.4, 19ab: 1.3: 14: 3.6, 7: 2.9, 9: 3.6, 12ab: 4.8. All of the oligonucleotides of the **5(cap)**, 17, 16, 8, 22, 20a, 11, 4, 20b, 15, 2, 10, 21, 18, 6, 3, **13**, **23**, **19ab**, **14**, **7**, **9**, **12ab**, **C**, **Poly(A)** (ref. 2).

Quantitation of the radioactivity in each major oligonucleotide (ref. 24) of the 20–22S PRB RNA gave the following near equimolar amounts expressed as c.p.m. per phosphate: 5(cap): 3.5, 13: 2.5, 23: 3.4, 19ab: 1.3, 14: 3.6, 7: 2.9, 9: 3.6, 12ab: 4.8. All of the oligonucleotides of the two subgenomic RNA species are derived from the 3' half of the viral oligonucleotide map except for oligonucleotide no. 5. We must, however, emphasise that the order of the oligonucleotides of the subgenomic RNA species cannot be deduced from our data, except that of the 5'-terminal cap-oligonucleotide, no. 5. This oligonucleotide was recovered from 20–22S RNA (Fig. 2b) and shown to contain the cap-structure (Fig. 2e) and to have the same RNase A-resistant fragments as the cap-oligonucleotide of virion RNA<sup>14,15</sup> (data not shown).

The presence of the 5'-terminal cap-oligonucleotide of virion RNA in subgenomic viral RNA species could reflect a splicing mechanism for the synthesis of subgenomic viral RNAs. Alternatively the viral genome could contain internal copies of this oligonucleotide. To distinguish between these alternatives we have analysed the 20–22S viral-specific RNA from cells infected by two recombinant RSVs. These recombinants have essentially indistinguishable oligonucleotide maps except for their 5'-terminal cap-oligonucleotides<sup>15</sup>. PR-A × RAV-2 no. 3 contains a RAV-2-derived cap-oligonucleotide, which chromatographs with hexanucleotides in a two-dimensional fingerprint<sup>15</sup>. The T<sub>1</sub>-oligonucleotides detected in the 20–22S intracellular RNA of this recombinant (Fig. 2c) are those shown in bold face type in the following oligonucleotide map of the viral RNA (ref. 2): **RAV-2-cap**, 15, 16c, 10, 12, 19, 21, 4, 20, 3, 33, 18, 11, 23, **5**, **7**, **2**, **8b**, **13**, **17ab**, **14**, **16ab**, **9**, **8a**, **C**, **poly(A)**. The c.p.m. per phosphate of the major oligonucleotides were: 15: 9.1, 11: 4.7, 5: 7.0, 7: 6.0, 2: 5.2, 8ab: 4.5, 13: 8.9, 17ab: 4.4, 14: 8.9, 16ab: 6.7, 9: 7.8. The presence of the cap-structure in the oligonucleotide-spot marked 'cap' in Fig. 2c is shown in Fig. 2f. PR-A × RAV-2 no. 5 contains a PR-A-derived cap-oligonucleotide, that is, no. 6, which is identical to that of PR-B (ref. 15). (Note, the map location of no. 6 has been revised. It was originally confused with no. 9, [ref. 2].) Figure 2d shows that the 20–22S intracellular RNA of recombinant no. 5 contains the cap-oligonucleotide no. 6 and all those oligonucleotides of virion RNA which are shown in bold face type in the following map (ref. 2): **6-cap**, 15, 16c, 10, 12, 19, 21, 4, 20, 3, 11, 18, **7**, **5**, **2**, **8b**, **13**, **17ab**, **14**, **16ab**, **8a**, **9**, **C**, **poly(A)**. The c.p.m. per phosphate of the major oligonucleotides were: 6(cap): 8.2, 15: 12.5, 7: 5.4, 5: 4.5, 2: 4.0, 8ab: 11.7, 13: 7.7, 17ab: 3.4, 14: 6.2, 16ab: 15.0, 9: 12.8. The 20–22S species of both recombinants also contained RAV-2 oligonucleotide no. 15, which maps near the 5' end of the virion RNA but does not contain the cap-structure. Thus, because the 5' cap-oligonucleotides of the 20–22S RNA species of PR-B, PR-A × RAV-2 no. 3 and no. 5 were identical to cap-oligonucleotides of their

print of 27–29S RNA of PR-B infected cells. (b) 20–22S RNA of PR-B infected cells. (c) 20–22S RNA of PR-A × RAV-2 no. 3 infected cells. (d) 20–22S RNA of PR-A × RAV-2 no. 5 infected cells. All numbered oligonucleotides were identified as identical to virion RNA counterparts (see text). Panels E and F are autoradiographs of RNase A, T<sub>1</sub>- and T<sub>2</sub>-resistant fragments of oligonucleotide spots expected to contain 7mGpppGmC(cap)-structures, after electrophoresis on DEAE paper at pH 3.5 (ref. 15). e, The nuclease-resistant fragments of oligonucleotide no. 5 of 20–22S PR-B RNA, recovered from the fingerprint shown in (b). f, The nuclease-resistant fragments of the oligonucleotide spot, marked 'cap' of 20–22S PR-A × RAV-2 recombinant no. 3 RNA recovered from the fingerprint shown in c.

RNases T<sub>1</sub>, T<sub>2</sub> and A after hybridisation with an excess (10–50-fold) of viral cDNA. These RNA pools were digested

respective virion RNAs, and the 20–22S of the two recombinants also contained a unique internal oligonucleotide (no. 15) that maps close to the 5' end of each recombinant RNA, the 5' segment of each 20–22S RNA species must have been transcribed from a proviral DNA segment corresponding to the 5' end of virion RNA.

A minimum estimate for the length of the 5' segment is 16 nucleotides (oligonucleotide no. 15, ref. 2) plus 22 nucleotides (large cap-oligonucleotide) or 37 nucleotides. This segment, however, is likely to be longer because recombination occurs between oligonucleotide no. 15 and the 5' end (as in recombinant no. 5) and because oligonucleotide no. 15 also occurs at the 5' end of RSV-B77<sup>12</sup> though it is not found in the first 110 nucleotides as sequenced by Shine *et al.*<sup>19</sup>.

It may be argued that the viral 5' oligonucleotides in the 20–22S cellular species were derived from minor contaminants of intact or degraded virion RNA selected by our cDNA rather than from a subgenomic 20–22S RNA. Although, our cDNA contained typically (ref. 23) three- to five-fold more sequences complementary to the 5' oligonucleotides than to other oligonucleotides (determined by hybridisation to an excess of <sup>32</sup>P-viral RNA and fingerprinting of RNase T<sub>1</sub>-resistant hybrids), this possibility has been ruled out for the following reasons (data not shown). The fingerprints of 35–40S viral RNA isolated from infected cells had an equimolar representation of all oligonucleotides indicating no bias in the selection process. In addition, if our PR-B poly(dC) cDNA was hybridised to a 10-fold excess of 30–40S <sup>32</sup>P-viral RNA and the hybrids were selected and fingerprinted as in Fig. 2 all PR-B oligonucleotides were approximately equimolar. This is because asymmetrical hybrids consisting of small, approximately 5S cDNA and large viral RNAs sedimenting between 10 and 35S, with a peak at 18S, were selected for fingerprint analysis by our method. Since the cellular viral RNA species analysed in this paper were also large after isolation (average approximately 18S), oligonucleotides adjacent to the 5' terminus should have also been detected if the 5'-terminal oligonucleotides were derived from contaminating intact or degraded viral RNA species.

We conclude that RSV-infected cells contain specific viral RNA species which consist of a capped segment from the 5' end of virion RNA attached to a polyadenylated longer segment from the 3' end. This suggests that subgenomic RSV RNAs are synthesised by a splicing mechanism analogous to that observed in adenovirus or perhaps by transcription from specifically deleted proviral DNAs. The data argue against subgenomic RNAs being generated by cleavage of 35–40S RNA or by internal transcription of full-length provirus. Further work is necessary to establish whether these subgenomic viral RNAs function as mRNAs.

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1. Baltimore, D. *Cold Spring Harbor Symp. quant. Biol.* 39, 1187–1200 (1975).
2. Wang, L. H., Duesberg, P., Mellon, P. & Vogt, P. K. *Proc. natn. Acad. Sci. U.S.A.* 73, 1073–1077 (1976).
3. Wang, L. H., Galehouse, D., Mellon, P., Duesberg, P., Mason, W. S. & Vogt, P. K. *Proc. natn. Acad. Sci. U.S.A.* 73, 3952–3956 (1976).
4. von der Helm, J. & Duesberg, P. H. *Proc. natn. Acad. Sci. U.S.A.* 72, 614–618 (1975).
5. Kerr, I. M., Olshevsky, U., Lodish, H. G. & Baltimore, D. *J. Virol.* 18, 627–635 (1976).
6. Stacey, D. W., Allfrey, V. G. & Hanafusa, H. *Proc. natn. Acad. Sci. U.S.A.* 74, 1614–1618 (1977).
7. Pawson, T., Harvey, R. & Smith, A. E. *Nature* 268, 416–420 (1977).

8. Hayward, W. S. *J. Virol.* 24, 47–63 (1977).
9. Bishop, J. M., Deng, C.-T., Mahy, B. W. J., Quintrell, N., Stavnezer, E. & Varmus, H. in *Animal Virology* (eds Baltimore, D., Huang, A. S. & Fox, C. F.) 4, 1–20 (Academic Press, New York, 1976).
10. Chow, L. T., Gelinas, R. E., Broker, T. R. & Roberts, R. J. *Cell* 12, 1–8 (1977).
11. Berget, S. M., Moore, C. & Sharp, P. A. *Proc. natn. Acad. Sci. U.S.A.* 74, 3171–3175 (1977).
12. Wang, L. H., Duesberg, P., Beemon, K. & Vogt, P. K. *J. Virol.* 16, 1051–1070 (1975).
13. Wang, L. H. & Duesberg, P. *J. Virol.* 14, 1515–1529 (1974).
14. Beemon, K. L. & Keith, J. M. in *Animal Virology* (eds Baltimore, D., Huang, A. S. & Fox, C. F.) 4, 97–105 (Academic, New York, 1976).
15. Wang, L. H., Duesberg, P. H., Robins, T., Yakota, H. & Vogt, P. K. *Virology* 82, 472–492 (1977).
16. Friedrich, R., Kung, H.-J., Baker, B., Varmus, H., Goodman, H. & Bishop, J. M. *Virology* 79, 198–215 (1977).
17. Coffin, J. & Haseltine, W. *Proc. natn. Acad. Sci. U.S.A.* 74, 1908–1912 (1977).
18. Collett, M. S. & Faras, A. J. *Proc. natn. Acad. Sci. U.S.A.* 73, 925–932 (1976).
19. Shine, J., Czernilofsky, P., Friedrich, R., Bishop, J. M. & Goodman, H. M. *Proc. natn. Acad. Sci. U.S.A.* 74, 1473–1477 (1977).
20. Gilham, P. T. *J. Am. Chem. Soc.* 86, 4982–4985 (1964).
21. Coffin, J. M., Parsons, J. T., Rymo, L., Haroz, R. K. & Weissmann, C. *J. molec. Biol.* 86, 373–396 (1974).
22. Junghans, R., Duesberg, P. & Knight, C. A. *Proc. natn. Acad. Sci. U.S.A.* 72, 4895–4899 (1975).
23. Cashion, L. M., Joho, R. H., Planitz, M. A., Billeter, W. A. & Weissmann, C. *Nature* 262, 186–190 (1977).
24. Beemon, K., Duesberg, P. & Vogt, P. K., *Proc. natn. Acad. Sci. U.S.A.* 71, 4254–4258 (1974).

## Errata

In the letter 'Structures of benzo(a)pyrene-nucleic acid formed in human and bovine bronchial explants' by A. M. Jeffrey *et al.*, *Nature* 269, p. 348, lines 25–26 in Fig. 1 legend should read . . . poly (G) were detected by ultra-violet absorbance at 280 nm and *in vivo* samples by their radioactivity. Line 19 in Fig. 2 legend should read . . . *in vivo* product was obtained (upper panel, *b*) which, when reanalysed . . . Line 2 in Fig. 3 legend should read . . . corresponding to the major *in vivo* DNA adduct from human and . . . Line 9 in Fig. 3 should read . . . (shaded area at 350–360 nm, <sup>1</sup>L<sub>b</sub>, short axis; 310–355, <sup>1</sup>L<sub>a</sub>, long. . .

In the letter by R. G. Strom and D. E. Harris, *Nature* 269 581–582 (1977), the title should read 'HD26676: Radio emission from a normal star'. On p. 581 paragraph 3 line 12, for 'Westerbok' read 'Westerbork'. In paragraph 4 line 5 for 'frequency  $\alpha$ ' read 'frequency<sup>a</sup>'.

In the letter 'Long-range attraction between red cells and a hydrocarbon surface' by D. Gingell and I. Todd, *Nature* 268, p. 767, two lines have been omitted. Line 12 in paragraph 2 should read . . . the oil/water interface was measured by the hanging drop. . . Line 28 in paragraph 4 should read . . . of attraction from 140 nm to 450 nm. The only kind. . .

## Corrigenda

In the letter 'Structures of benzo(a)pyrene-nucleic acid formed in human and bovine bronchial explants' by A. M. Jeffrey *et al.*, *Nature* 269, p. 348, in Fig. 2 legend line 18 for 50–60% methanol read 50–65% methanol.

In the letter 'Surge activity on the Barnes Ice Cap' by G. Holdsworth, *Nature* 269, p. 588, the journal in ref. 14 should be *J. appl. Met.*

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# christmas Books supplement

## Images of animals

Colin Blakemore

*Animals and Men: Their Relationships as Reflected in Western Art from Prehistory to the Present Day.* By Kenneth Clark. Pp. 240. (Thames and Hudson: London, 1977.) £10.50.

EVERYTHING about this book is full of promise. Its author, Lord Clark, is now a cult object more widely revered than Horus and Hathor, the sacred falcon and cow of ancient Egypt who decorate several of the book's ample pages. The title is provocative and challenging. The book is (literally) weighty and the many, many illustrations are generally of high quality. Most of all, the timing is impeccable. It is surely no accident that this elegant volume appears a few weeks before Christmas in a year of intense public interest in the status of animals and the need for their protection. Not only is there the growing public sentiment for the conservationist movement, but there is also heightened anti-vivisectionist activity in response to the centennial of the British Cruelty to Animals Act. There can be no more convincing sign of the times than the growing power of the 'Ecology' movement, even in France—that bastion of effortless pragmatism, where there has always been more appetite for, than love of, animals.

Despite the richness of its potential and the certainty of its commercial success, the book in actual fact does not fulfil its promise. Lord Clark's text is merely a brief essay, which at times is no more than a list of the plates that follow it. The captions to the illustrations, which enlarge a little on the text, were not even written by Lord Clark but by three assistants. Most disappointing of all is the fact that the book is *not* primarily about the relationship between animals and man: it is largely a catalogue of western works of art that happen to contain images of animals.

Text and plates are arranged around five subjects: "Sacred and Symbolic Animals"; "Animals Observed"; "Beauty and Energy of Animals"; "Animals Beloved"; and "Animals Destroyed". A scholar of Lord Clark's academic stature, vast experience, and skill in communication is expected to bring insight and even humour to these topics. And he does. The balance between admiration, curiosity and love in our relationship with animals is nicely handled. And there are fascinating vignettes—like the fact that Leonardo was

a vegetarian; that Cezanne, Monet and Manet never painted an animal; and that for 700 years all animals in art were symbols for the evangelists.

But the closest that Lord Clark comes to deeper issues is on the very first page, where he asks why the mythical harmony



of animals and man in the Golden Age never actually came to pass. "The answer lies in that faculty which was once considered man's highest attainment, a gradual realization that the sounds he uttered could be so articulated as to describe experience. He discovered words, he could communicate with other men."

This idea (that language bluntly separates man and beast) recurs from time to time. To the Egyptians, the haughty speechlessness of animals made them worthy of worship. And we are said to play with animals because in doing so "we forget the difference that separates us—the faculty of speech. Children would rather play with a teddy-bear than with a small model human being, and they put long imaginary speeches into the bear's mouth. Thus, the barrier between animals and man is broken down". Quite apart from the fact that Lord Clark does not seem to have heard of dolls, I do not trust the hypothesis that people are truly distinguished from animals by their speech, any more than the suggestion that the relationships between individual human beings rest on language alone.

What of the remarkable communion that can exist between a rider and his horse, between a farmer and his herd,

between people and their pets? And, equally, what is it that makes us wince at the mindless existence of a battery hen or bristle with anger when we see magnificent animals languishing in tiny cages? I believe that our extraordinary empathy with animals springs from the fact that we are animals too. Animals with insight enough to recognise our biological oneness with other beasts.

And yet our animal nature, as well as making us admire and understand the special skills of other species, also drives us to kill and eat a number of them, because that is what we, as animals, are clearly designed to do. Lord Clark certainly touches on this paradox in our attitude to animals. "We love animals, we watch them with delight, we study their habits with ever-increasing curiosity; and we destroy them." But he does not face squarely the question of man as an animal of such cultural sophistication that he can use a representation of another species as an exercise in the exploration of his own deepest emotions.

Even as an animal picture-book, it has some surprising omissions. Where are Henri Rousseau's strange jungle beasts with their haunting eyes; the mysterious animal automata of Maurits Escher; and the folksy farmyard beasts of Marc Chagall? But more important, where are the works of art that explore the real question of man as animal? Goya's terrifying masterpiece of *Saturn Devouring his Children*, the Tiepolo *Martyrdom of St John of Bergamo* (what a chance to compare it with the Delacroix or Rubens' *Lion Hunts*); the mythical man-beasts of Michael Ayrton; and the lumps of animal flesh that pass for portraits by Francis Bacon?

Lord Clark explains in a Foreword that this enterprise grew out of a commission for a book on animals in art from the World Wildlife Fund (which will receive a contribution from its sales). "This by itself did not seem to me to constitute a subject," writes Lord Clark, "but on reflection it occurred to me that no-one had ever given much thought to the relationship of animals and men, and that this might be a subject worth exploring." Of that there is no doubt. Let us hope that it will continue to be explored as part of the effort to establish the true nature of man.

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## Birds of prey, owls and seabirds

WHY birds of prey should be so popular is rather difficult to fathom, for they are not exceptionally popular with bird-watchers and there are many bird-lovers and perhaps shooters who would think of them as "cruel killers". Perhaps supporters of birds of prey are just prolific writers, for each year they become the subject of several books. Last Christmas three books came to me for review; this year, they have retained their popularity with another three. Sometimes, one finds the same sort of text with the same facts and theories possibly presented in a slightly different way. Whether the texts are original or 're-hashes' depends on how far into the basic papers the popular writer is prepared to read. Two of the three books on birds of prey in this selection are written by well established raptor enthusiasts: Michael Everett writes on the *Natural History of Owls* (Hamlyn: London and New York; £3.50) and Lea MacNally provides an individual account of *The Ways of an Eagle* (Collins: London; £5.95).

The *Natural History of Owls* is a 'popular', large format book, well written

and well researched, with many photographs chiefly in black-and-white: there may be some sense in this because plate after plate of brown and grey owls tend to become monotonous anyway. Also, most owls conform to much the same physical pattern of a non-specialist predator, which are most highly developed towards living in the dark. Everett provides particularly good reviews of the chief hunting methods used by owls, of which there are relatively few, and of the work undertaken by many authors on the feeding ecology of a number of species. He also summarises the known complexities of the predator-prey cycle; these tend to become ever more complex where the bird's range covers many habitats. Studying nocturnal birds is particularly difficult, even though "image intensifiers" have occasionally been brought into use for certain aspects of research. The final chapter "Owls and Man" returns to a theme which has been much worked on over the years—their effect on superstitious Man. The author traces Man's reactions to owls from the time when the owl was regarded as a welcome neighbour, to the time of the so-called agricultural revolution in the mid-eighteenth century and the rise in field sports, when all hook-beaked birds, regardless of their known food preferences, were destroyed as enemies. It is only recently that scientists of the Nature Conservancy Council, the Institute of Terrestrial Ecology and the Royal Society for the Protection of Birds have begun to show with incontrovertible data how misleading have been the prejudices of some of the shooting landlords and their gamekeepers. In short, this book is a well written and easily read summary of what is known of the owls of the world.

Lea MacNally's *The Ways of an Eagle* is different in that it is based on personal experience with several pairs of eagles gained over 22 years as he followed his profession, first as a deer-stalker and latterly as a Warden of the National Trust for Scotland in north-west Ross-shire. The book is divided into four sections which deal with various aspects of the life history of the four pairs of eagles on which he had concentrated. The sections deal with the survival of the golden eagle, nesting and fledging, their food, behaviour throughout the season, and finally, four appendices detailing breeding success, weights of eaglets, lists of prey and nest material brought to the nest. One advantage that this type of book has over the monograph is that the real personal experience shines through, so that you get the feeling that you are really with MacNally in the hills. You savour more clearly the thrill of seeing these huge eagles, for which Britain is one of the most important—if not the most important—stronghold in the world.

Even though MacNally concentrates on the golden eagle this book is not a mono-



Great horned owl

Taken from *Natural History of Owls*

graph: he has not collected or attempted to synthesise the results of the enormous amount of research which has been undertaken on the golden eagle. Notwithstanding this, Lea MacNally gives us a very readable account of the ways of the golden eagle.

*The Birds of Prey in Britain and Europe* (Hamlyn: London and New York; £1.50) by Miroslav Bouchner and Dan Barta is a concise guide in colour, with a refreshingly different text written by a Czechoslovakian and presumably based on different sources from that available to English writers. The illustrations perform their function and enable one to identify raptors when observed in ideal conditions. There are also two useful plates showing the typical underviews of most species, but these do not of course indicate the range of plumage variation possible with birds of prey. Unfortunately, this book has no bibliography; so it would be difficult for the serious raptor enthusiast to trace statements to their original source.

Seabirds are another group of birds which have their adherents, and to cope with a popular demand from visitors to Britain's coastline Bruce Campbell has written *Birds of the Coast and Sea: Britain and Northern Europe* (Oxford University: Oxford; £3.75), which has been illustrated by 64 paintings by Raymond Watson. Campbell's text accompanies the paintings closely but he has also added shorter notes to describe the appearance and habits of the less common species which could appear but which have not been illustrated. Each specific text begins with a description of the plumage of each species, information about their geographical range and breeding distribution, their calls, breeding behaviour, size of population, and finally anecdotal accounts of interesting aspects of a bird's behaviour. The whole is well written and should provide ornithological companionship.

The illustrations do not reach the same standard as the text. The style of painting is representational, as far as the birds are concerned; Mr Watson does, however, avoid painting a bird's habitat in the same detail and so the bird appears on a misty shore lit by subdued light. He does not

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always get the shape of the bird quite right and often draws the bill rather too long. I think I might have trouble in trying to identify some of the small waders from these pictures but one or two of the pictures of gulls in flight have light, life and beauty.

My final comment on this book is that it is sad to see the text printed in such small type. Presumably it was used in a laudable attempt to keep the price down—which is reasonable as present prices go.

Another book which is not easy to read is the large format book with several photographs on each page. In *Wonderful World of Birds and their Behaviour*, by Donald Broom (Hamlyn: London and New York; £2.95), I get the impression that the designer was thinking more about designing the page to sell the book by its photographs than about the difficulty the reader might have in finding his way to the text through the jumble of pictures and legends. This lay-out I found particularly distracting, even though Donald Broom has written a text which gives a good succinct general description of different aspects of the way that a bird lives, beginning with a chapter on the physical characteristics of some of the more exciting birds of the world. This is followed by chapters on their evolution and a description of the families of birds, how they feed, their courtship and nesting, migration, and finally a chapter on the economics of birds as they are used by man or as pests. The colour photographs are excellent and as a general rule the legends are informative.

*Penguin* (Peter Owen: London, 1977; £5.95) is another seabird title, but the book is a personal account by L. Harrison Matthews apparently written from his 50-year-old diaries about a visit with the *Discovery* expedition of 1924 to the Antarctic, on which he was primarily engaged in whale research. There are six chapters, five of which are headed by the names of various penguins and one chapter entitled Captain James Cook. The text is a mixture of anecdotes of early travellers in the far south, personal reminiscences of his own life and voyages, and of the birds encountered on them. The ornithological interest is rather thin, comprising extracts from his diaries about the birds he met 50 years ago and occasionally referring to more up-to-date work. The photographs were apparently taken at the same time. More recently taken and clearer photographs might have helped the layman, for whom the book must have been intended, with the identification of the species referred to in the text. It is a pity that the author did not stick to the subtitle *Adventures among the Birds, Beasts and Whalers of the Far South* as the main title for, as a personal account of his travels in the far south, this is an amusing book to read. The title *Penguin* is misleading.

Whereas most of the books that I have been reviewing have been about the results of other people's research, there is only one book which described the techniques of watching birds. In this case, as its name *Bird Count* (Kestrel/Penguin: Harmondsworth, UK, 1977; hardback £2.75; paperback 75 pence) suggests, it summarises the various methods which have been developed largely by the British Trust for Ornithology for their surveys and censuses of the bird populations of various habitats in Britain, in which they have involved a large proportion of their membership. This book is intended for



Puffins

Taken from *Birds of the Coast and Sea*

anyone interested in discovering more about wild birds in a methodical and thorough way. Humphrey Dobinson begins with a chapter identifying about 60 common birds, which I would have thought was slightly out of place; anyone who was going to use the techniques that followed would need to have had far more experience at identifying birds before embarking on the surveys. He follows with chapters on the various methods of counting birds. The common bird census

has been an invaluable technique in showing changes in the population levels of breeding birds since 1962. The technique was originally introduced at a time when many of Britain's birds were dying as a result of the misuse of the organochlorine pesticides and when it was felt that the bird population needed to be monitored on an annual basis. The author gives a summary of some of the results of the first ten years of the survey. Particularly noteworthy was the discovery that several species of birds which bred in this country were severely hit by the disastrous droughts in the Sahel region, and population levels of several species, notably whitethroat and sand martin, were considerably reduced. The count also shows that the populations of many wintering species, goldcrest amongst others, have soared in recent years because of the lack of severe winters. Dobinson then goes on to describe other techniques of counting birds on waterways, how to count colonial birds including seabirds, how to find nests, and how to study migration.

The book does not contain all the detail that a textbook would have, and anyone seriously needing to use these well-tried techniques would need to seek the original papers. There are also simpler methods of assessing the relative abundance of birds; this he does not mention. Mr Dobinson is a schoolmaster and his book was, I felt, written very much with the senior pupils and other amateur bird-watchers in mind, who may have passed beyond the listing and identification stage and want some project which will give them something more to think about. For this purpose, the techniques can produce useful results. Mr Dobinson has produced a very useful and serious book, which contains much useful information.

Peter Conder

Peter Conder recently retired as Director of the Royal Society for the Protection of Birds.

## Bird-watching in West Africa

*A Field Guide to the Birds of West Africa.* By W. Serle and G. J. Morel. (Collins: London, 1977.) £5.95

THIS new *Field Guide* should prove indispensable to the growing number of bird watchers who visit West Africa. The area covered includes the entire bulge of Africa south of the Sahara and extending eastwards to Chad and the Central African Republic. The text is clear and concise, and contains many useful nuggets of information: how to tell, for instance, the difference between the African golden oriole and its European counterpart, both of which may be in the same area at certain times of the year.

The coloured illustrations are, of course, vital to amateur and professional ornithologists, and most of them are reasonably accurate, though a few are perhaps a shade too bright. It is a pity that the birds of prey are not in colour, as this group of birds is notoriously difficult and confusing to identify. The picture of the mouse-brown sunbird (opposite p225) shows a typical down-curved beak, whereas the description (p229) clearly states that this sun-bird has a short, straight beak.

These points aside, however, and bearing in mind that the book has around 1,000 birds to deal with, this *Field Guide* reaches the high standard which ornithologists all over the world have come to expect from Collins.

J. & G. Newmark

J. & G. Newmark have recently returned from an ornithological field excursion to West Africa.



## All the world's a stage

*Manwatching: A Field Guide to Human Behaviour.* By Desmond Morris. (Jonathan Cape: London; Abrams: New York, 1977). £7.95; \$16.95.

"ALL animals", says Desmond Morris, "perform actions and most do little else". And all popularisers of ethology, he might have added, utter platitudes—and most do little else. The problem with his *Field Guide to Human Behaviour* is not so much that it's simplistic (which it is) or misleading (which it is) as that it's astonishingly trite.

It is trite and at the same time demeaning. If a zoologist is going to turn his binoculars—or opera glasses—on human beings he must get them the right way round; otherwise the people under study become manikins, the "field" of human behaviour becomes the land of counterpane, the Human Comedy a puppet show. Morris, here as never in his earlier books, comes over as an out-and-out reductionist. Animated little men and women trip across his stage, selfishly pursuing the goal of their own biological survival, pair-bonding and pair-breaking, staking out territory, hunting the Sunday lunch; they wave to each other with one of the three types of wave, they show their friendship with one of the 14 types of tie-sign; they have their exits and their entrances. Most of the time they use clothing signals to cover these exits and entrances from view.

Morris claims that as a "manwatcher" he is simply doing for people what bird-watchers do for birds: he records their behaviour and attempts without bias to provide historical or functional explanations for it. But when men watch birds they are observing a foreign species; they can assume no privileged knowledge of what the birds are doing or why. When men watch men, however, (as perhaps when birds watch birds) such an assumption of initial ignorance is at best tedious and at worst insulting to common sense. If a bird was to read this book, it might well find many of Morris's observations novel and intriguing. A person reading it, however, is certain to be less impressed: he must find much of the material boringly familiar (because he has been a manwatcher as long as Morris has), he must be irritated by the pseudo-scientific classification of behaviour patterns (because he guesses that they are largely Morris's invention), and he must realise that many of the biological explanations are plain silly (because he cannot believe, for example, that "viewed biologically, the modern footballer is revealed as a member of a disguised hunting pack").

That said, there are nonetheless places in the book where the old Morris whom we knew and (some of us) loved takes over. Hidden in the bran, there are a few real prizes. Yet even when Morris does say

something thought-provoking, the effect is lost because he fails to follow through. For reasons best known to himself and his editor, he quotes no authorities and no sources of evidence (the reading list at the back of the book has no immediate bearing on the text). The result is that all his more interesting assertions are left hanging in thin air. An informed reader may of course sometimes recognise where the ideas come from, but when he does so he will not always be reassured. For Morris is indiscriminating in what he is prepared to relay as established scientific fact: he has lifted some good ideas from the scientific literature, but he has lifted a lot of rubbish too.

If people get little from reading the book, they may still find pleasure in looking at it. It is beautifully and informatively illustrated. Indeed, the pictures often succeed where the words fail in giving insight into how human beings behave. Again and again, the evidence of particular photographs belies the stilted text. Here are real people fighting, loving, praying to God... The camera, framing a moment of their lives, calls us to attend to things which we half knew but never waited to consider. Meanwhile, Morris calls us to attention, tells us what to think: "Religious displays are essentially submissive gestures performed towards a super-dominant figure—the deity..." "More intimate than the Hand-in-Hand is the Waist Embrace (top



Female sporting behaviour

Taken from *Manwatching*

right). Because it involves close trunk contact and impedes locomotion, it is usually employed by strolling couples with time to spare..." "The butcher's shop has become the modern hunting ground, but now it is the woman who carries home the 'kill', while her male engages in symbolic hunting called 'work'". In his own words: "Some individuals find the wink difficult to perform even as adults".

**Nick Humphrey**

*Nick Humphrey is Assistant Director of Research in the Sub-Department of Animal Behaviour at the University of Cambridge, UK.*

## Animal collections in the UK

*Animals on View.* By Anthony Smith. (Weidenfeld and Nicolson: London, 1977.) £6.50.

A GOOD guide should be concise, accurate and up-to-date, at least at the time of publication. Anthony Smith, in his extremely well illustrated guide to Britain's safari parks, zoos, aquariums and bird gardens, succeeds in achieving these requirements most successfully, and the general user will have little to complain about. The eyebrows of the regular zoo user may be raised a little over one or two caption errors which make parakeets of love birds and American bison from European ones.

It should not be imagined that the guide takes a clinical approach to the subject. Anthony Smith has done too many exciting things elsewhere to allow this to happen. He describes the factual details of the collections without turning it into a checklist of all the flora and fauna, but emphasises those aspects of breeding, exhibition and use for which individual zoos are famous. He also goes on to assess the management of some of the larger collections and in one or two cases he accurately predicts their closure. His assessments, which are of course the impressions gained on his visits to all the major collections in the guide, add another

interesting dimension to the work.

Anthony Smith's introduction to the book covers most aspects of animal collection management and the problems which can arise. I think he is very right to bring out these points, although one gets the feeling that here is the embryo of another book, which hopefully will be forthcoming, as the introduction is bound to leave very large question marks in many readers' minds.

His bias towards safari parks does shine through a little too brightly for the average "zoo man", but this is perhaps inevitable from someone who has been fortunate enough to spend much time in the wild places of the world and who is perhaps more aware of space in an ethereal sense than in terms of practical animal management. There are good zoos and good safari parks in terms of how much regard they give to the welfare of their animals. Sadly there are also bad ones in both categories.

The work is an important addition to any regular zoo user's bookshelf, and I believe that the more casual of zoos' twelve million or so annual visitors would obtain considerable interest and insight into the enormous variety of creatures that may be seen in collections, large and small, throughout the length and breadth of the UK.

**R. J. Wheeler**

*R. J. Wheeler is Director of the Royal Zoological Society of Scotland, Edinburgh, UK.*



## African wildlife

PICTURE BOOKS about African wild animals suffer a population explosion inversely proportional to the decline in the populations of their subjects. All the arts and crafts of photography and book design combine in sharpening their competition for dominance in the face of the selective pressure of the bookshop browser, who is himself in danger of being bewildered by the plethora of jostling titles.

Some books are the outcome of scientific studies on the lives of various large animals, and are valuable contributions to knowledge made by naturalists who, surprisingly, seem now to prefer being called ecologists, ethologists, and so on, as a fancied boost to their dignity—Linnaeus, Gilbert White, Darwin, Wallace, Bates and the other giants of the past needed no such props.

Most of the books, however, are essentially picture books, often of the highest photographic standard, showing the beauties of tropical scenery and animal life. They concentrate on the eye-catching and spectacular parts of the flora and fauna, but give little information about them beyond the "visual display".

The third class is that of the doomsters bewailing the fact that the frontiers of the wilderness are steadily receding in the face of ever-growing human occupation and development of the land. Much as the loss may be deplored, it is a fact that will not go away; wildlife is rapidly becoming confined to reserves and parks which, as time goes on, will become more and more artificial under management and conservation. Only those species such as the jackal in South Africa, and the fox and badger but not the otter in Great Britain, that can survive persecution by man, will remain as wild animals. Even now the average package tourist, conducted to viewing points, and sheltered in hotels or lodges with all European amenities so that he is protected from any close contact with the wild, sees little more than he does in a 'Safari Park' at home—only the scale is larger. The very word 'safari', used by the exploiters to beguile him with romantic overtones, merely means a caravan of beasts of burden, or a journey.

*The White Lions of Timbavati*, by Chris McBride (Paddington: London, 1977; £5.95) is the by-product of a scientific study on the lives of a group of lions that McBride kept under observation for about a year in a large private nature reserve in the north-eastern Transvaal. The group consisted of two dominant males sharing half a dozen lionesses accompanied by their cubs and adolescent offspring. One of the lionesses produced a couple of white, but not albino, cubs, on whose birth and early life the story concentrates. Later, another white cub was born to a different lioness, and the author thinks the white

births were a result of the frequent matings between father and daughter or grand-daughter, so that homozygotes for the recessive white gene appeared. The white cubs were handicapped by their lack of concealing colouration, and probably survived only because McBride provided them with food at critical times. His book tells of his adventures with his wife and small daughter living in a bush camp while studying the lions, and makes an excellent read. He has a pleasant straightforward style of writing, and in addition to his narrative he sets out his common-sense thoughts on wildlife conservation and management.

*Savage Paradise*, by Hugo van Lawick (Collins: London, 1977; £13.95), is a picture book. Van Lawick claims to be a



Lions

Taken from *Back in the Wild*

cameraman first and only secondarily a naturalist. His large coloured photographs, up to 20 by 14 inches in size, illustrating the lives of the predatory animals of the Serengeti plains and their prey, are unsurpassed in subject and composition. The author hopes that his pictures will reflect his love for the Serengeti and the Ngorongoro Crater; "but in an honest way, for with its beauty there is also harshness, a savage struggle to survive in a paradise." *Savage* indeed is the picture of half a dozen hyaenas tearing out the entrails of a living zebra; in spite of the zebra's facial expression, the author assures us that "it is doubtful if the prey feels much pain", a thought that may comfort him if he ever finds himself in a similar predicament. He prefaces his book with informative essays on each of his subjects—lions, leopards, cheetahs, hyaenas, jackals, and wild dogs—detailing his experiences with each species and "some of the remarkable things that happened while I was watching them." This is a superb book of its class.

*Pyramids of Life*, by John Reader and Harvey Croze (Collins: London; Leppincott: Philadelphia; £6.95; \$12.95), is a very different book. Although every page is adorned with splendid photographs taken by Reader, the pictures are chosen to illustrate the text of each 'double spread' by Croze. The main theme of the book is the circular movement of energy through each

ecosystem, the cycle starting with the soil receiving sunlight, water and air to produce plants that are eaten by herbivores which in turn are eaten by the carnivores at the top of the pyramid. At any point in the ascent from primary producer to carnivore, the organisms may die, whereupon their bodies are reduced by mostly microscopic decomposers into simpler substances which return to the soil for the start of another cycle. All this may seem obvious enough, but the infinitely varied paths that the cycle can take, and the inter-relationships of the plants and animals concerned, as described and pictured here, will be a revelation to the intelligent layman who prefers to understand something of what he sees rather than merely making it the subject of holiday snapshots. In the epilogue, Croze points out how knowledge of the way the amounts of energy and materials determine the number of animal and plant pipelines, up through the pyramid of life and back down to the soil, can be used to preserve the richness and yield of terrestrial ecosystems; and that we should not be discouraged by the notion that natural beauty is not its own excuse for existence. More care in editing the book would have avoided such a solecism as 'an algae is', repeated twice.

*In Noah's Ark is Stranded*, by Björn Berglund (Macdonald and Jane's, 1977; £6.95), the author claims to deliver the "message of African ecology". Berglund is a Swedish journalist who wrote his book after visiting nine East African national parks, where he was shown all the usual sights. The accuracy of his observations on animals may be judged by the double-page photograph of a crocodile walking in a manner exactly the opposite to that stated in the caption. The author seems to suffer from the typical Swedish guilty conscience, for throughout his book he gives the main emphasis to bemoaning man's destructive impact on his environment in Africa. He tells us that "ecology teaches the dependence of the living organism upon the environment and their harmonious interaction", and concludes with a glimpse of the obvious in the admonition that "we must accept that we function within an environment", asking: "are we able to live in harmony with each other and our environment?". He need not look far for the answer.

*Back in the Wild*, by Sue Hart (Collins: London, 1977; £5.50), is a collection of short articles reprinted from a South African newspaper. Most of them are about conducted visits to various parks and reserves, where the author seems to have looked on the larger members of the fauna as people dressed up in animal skins. The articles are so slight, superficial, and often inaccurate as to fact, that one well may wonder why a publisher should bother to reprint them. The drawings by Mrs Leigh Voigt are better than the text.

L. Harrison Matthews

L. Harrison Matthews was Scientific Director of the Zoological Society of London from 1952–66.



## Ornamental creatures

CHRISTOPHER LEVER's exciting and scholarly book *The Naturalized Animals of the British Isles* (Hutchinson: London; £7.50) is full of surprises. Did you know that there are Mongolian gerbils on the Isle of Wight, Midwife toads in Bedford, Pumpkinseeds (a species of sunfish) near Tunbridge Wells and Wallabies in Yorkshire? These strange animals and many more are established in the wild in Britain in self-maintaining populations. Many of our more familiar animals have also been introduced by man, including the rabbit, brown rat, house mouse and grey squirrel. Fifty-nine species of vertebrates are described with a separate chapter devoted to each, in which the author describes exactly how, from where and when, the animal was introduced, and summarises its biology and the effects of the introduction on our native flora and fauna.

Many species, such as the pheasant, were introduced on purpose for their food value. Others were brought here for "economic reasons", like the little owl, imported from Italy by an eccentric who had the inspired idea that it would be a useful means of pest control in the kitchen garden and would also rid our church belfries of their sparrows and bats. The difficult question of the advantages and disadvantages of introducing foreign creatures is discussed. Few would deny that we would be better off without the rat; but for Christopher Lever, at least, life in Britain would be miserable without such ornamental creatures as the beautiful and harmless Mandarin duck, to whom he dedicates his fascinating book "with admiration and affection".

Introduced animals, however, can have disastrous effects. Feral dogs on the Galapagos Islands have gone wild and are decimating several of the island populations of land iguanas. A captive breeding project has been set up in an attempt to protect these magnificent beasts. This, together with many other conservation efforts by the World Wildlife Fund (WWF), is described in their yearbook *The World of Wildlife* edited by Nigel Sitwell (Hamlyn: London; £2.95). The book, which is illustrated with some superb photographs, contains sixteen chapters on endangered species, including the tiger, narwhal, whooping crane and desert pupfish, and describes the efforts that are being made to save them and their habitats. Thanks to the successful Operation Tiger campaign, launched by the WWF, reserves have been set up throughout India, and the tiger now has a good chance of survival.

The most dramatic success of the WWF conservation effort last year must be the Italian government's agreement to give

complete protection to the hundred or so remaining wolves in the Apennine mountains. And let us give a thought this Christmas to the beautiful scimitar horned oryx, now in grave danger of extinction. An important breeding centre has been set up in the Marwell Zoological Park in Hampshire, so perhaps we can look forward to the day that the oryx join the wallabies as naturalised British citizens?

I sometimes wonder just why it takes so long for new ideas in the scientific literature to filter through to the popular book market. *Inside the Animal World* by Maurice and Robert Burton (Macmillan: London; £6.95) is subtitled *An Encyclopaedia of Animal Behaviour*, and although it provides an extensive catalogue of behaviour, it leaves little room for explanations of how the behaviours could have evolved or what ecological circumstances favour certain behaviour patterns rather than others. Thus, we are told that some species of frogs are good parents though others are not; but the interesting question is why is there a difference?

The vital function of territorial behaviour is said to be the regulation of population numbers so as to prevent overcrowding. But I feel that the layman is entitled to an answer to the question of why the "doomed surplus" accept their miserable role in life. There is next to no reference to the fact that different individuals may be selected during evolution in different ways. How much more fascinating courtship behaviour becomes

when we realise that optimal reproductive strategies for males and females are rarely similar. Animals may often signal their sex "because it is a waste of time for a male to court another male", but in some instances it apparently pays a male to mimic female behaviour. Some male elephant seals pretend to be females (the "Danny la Rue strategy") and join a harem to steal copulations, unnoticed by the dominant male. Surely we can no longer be satisfied with explanations of courtship displays as "designed to overcome the fear of close contact", or of lions killing young fathered by other males as a result of a "feeding frenzy". Male lions can in fact enhance their reproductive success by such murderous behaviour, because this brings the females into oestrus again sooner and thus hastens the day that the male can sire his own offspring.

This is, however, an attractive book and will fascinate those readers who want to learn about how wonderful nature is, without wanting to know the reasons why. I feel that the lovely line drawings by Hilary Burn and the outstanding photographs by Jane Burton deserve considerably more acknowledgement than their brief mention on the flyleaf of the dustjacket.

Nicholas Davies

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## Desert habitats

IN spite of all the constraints desert conditions impose on human activity, they do generate in the individual a great sense of freedom. This, perhaps, explains why so many of those whose names are so closely linked with arid regions were, above all, great individualists. Arabian explorers like Lawrence, Philby and Thesiger have their counterparts in the New World—naturalists who, in their very different way, were no less individualistic and devoted to the desert habitat. Twenty years ago, two books of Edmund C. Jaeger (now in his nineties, and still enjoying the desert environment in Riverside, California) formed an excellent introduction to the North American deserts, particularly those of California. In the early sixties, Alonzo Pond wrote his highly individualistic account of deserts in *The Desert World*. In the same mould is Raymond B. Cowles. Born and raised in the South African bush, he subsequently spent nearly sixty years studying the adaptation of living organisms to desert conditions in Southern California. He pioneered work on reptilian thermoregulation; and his *Desert Journal*

(University of California Press: Berkeley, Los Angeles and London, 1977; \$10.95; £8.25) is a reflective account of the background to these studies. The work conveys his enthusiasm, insight and excitement at unravelling the nature of the behavioural and physical adaptations of desert animals to the lethal heat and aridity of their habitat. More than this, it gives the reader an appreciation of the total desert environment.

*The Desert*, by J. L. Cloudsley-Thompson (Orbis: London, 1977; £4.95), makes equally easy reading but is quite a different work. Connoisseurs of coffee-table culture may be forgiven if they recall similar offerings from other publishers (Time-Life, Aldus), some with the same title, some with similar photographic credits, some even with the same author. But readers can be assured that the text is different. Also, only in the volume under review are the cold deserts of polar regions considered in addition to hot deserts. At the price, this handsome volume is good value for the layman and widens his choice of introductory books from what must now be an almost saturated market.

M. J. Chadwick

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## Pictorial delights in marine science

THIS year has seen the publication of five books on various aspects of life in the seas and oceans. All are published to a high standard and are sometimes called 'coffee-table books', designed to attract the casual looker rather than the hard reader. All of them are intelligible to the non-scientist but some would be useful in sixth-form (advanced school) libraries or for general reading by undergraduates, thus giving a wide potential market.

Probably the most information is packed into *The Undersea* (Cassell: London; Macmillan: New York; £12.50; \$27.50), a book of grand dimensions edited by N. C. Fleming of the UK Institute of Oceanographic Sciences. It contains chapters by different authors on the ocean floor, the water itself, plant life, salt-water animals, the ocean resources, the use of ocean space, underwater archaeology, diving and divers, submarine craft, and marine law and politics. This is oceanology rather than oceanography. The book is expensive but the standard of production is high, with hundreds of black-and-white and coloured photographs and diagrams. A major criticism is that the legends for these are often inadequate and some of the diagrams are poorly conceived. Although some of the chapters are difficult, for example that on the ocean floor, most of them will be understood by the lay reader. There is a good index and modest bibliography.

The other four recent books are more limited in scope. In *The Seashore and Its Wildlife* (Orbis: London; £5.95), Robert Burton, a naturalist author, sets out to describe the nature of the shore, how it is formed, its variability in different parts of the world, and its flora and fauna. This delightful book is packed with excellent colour photographs, and the text is readable and informative, with a good index and bibliography. It must rate as a best buy in terms of production standard and price, if one wants a book on the shore, but I would rate it educationally only up to sixth-form level as general reading.

In a rather similar category is *Animals of the Oceans: The Ecology of Marine Life* by Martin Angel and Tegwyn Harris (Eurobook/Peter Lowe: London; £4.75), which contains many excellent photographs but also some poor and distinctly garish diagrams. There are chapters on the ocean environment, the ocean's fishes, the edge of the sea, coral reefs and mangrove swamps, plankton, the open sea, air-breathers, and man and the ocean. There is a short bibliography and glossary, and an index. This book is obviously good value for money; it is elementary in style but readable and

fairly informative. Perhaps the authors tried to do too much in the space available.

Finally, there are two books on marine mammals. In *The Encyclopaedia of Sea Mammals* (Hart-Davis, MacGibbon: London; £12) D. J. Coffey sets out to produce something approaching an encyclopaedia. There are three main sections: dolphins, whales and porpoises; seals, sea lions and walruses; and dugongs, sea cows and manatees. With each group there is an alphabetically arranged list of features on life history, ecology, exploitation, and so on, followed by a list of species, each briefly described. There are some indifferent diagrams, some really

identification. Both books bring out well the lore surrounding sea mammals, especially the Cetaceans, and they rightly point to the problems of conservation. I find their strictures on the use of dolphins in warfare less easy to understand. It seems to me that using dolphins to flush out enemy frogmen or carry limpet mines is little different from the widely accepted use of police dogs or horses.

When reviewing popular or semi-popular books of such a high standard of production, an inevitable comparison is made with the strictly technical literature, where £20–30 is a common price range.



The capture of a whale is seen as cause for general excitement in this Dutch sixteenth-century print. The workers' efforts are spurred on by the bagpipe player and flagbearer (left), while in the background less fortunate whalers fall prey to fearsome monsters. Illustration taken from *The Encyclopaedia of Sea Mammals*, reviewed on this page.

excellent photographs, and copies of prints and other pictures dealing with the subject. The academic standard is about sixth-form level. Although the book could be used to identify some of the species described, it is by no means a key to identification, and many of the photographs are taken for dramatic effect rather than recognition. At £12, this book is by far the highest-priced for its size. Perhaps the price is set high because the book is of more limited interest and the potential sales therefore lower.

*Mammals of the Seas* by R. M. Martin (Batsford: London; £4.95) is a book of similar length, style and standard to the Coffey book. The difference in price, however, is astonishing. Species are listed and there is a glossary, index and bibliography. It is a pity that neither book contains a really good key or section on

Presumably, such technical books sell 1000–5000 copies. What is the estimated sale for the books under review? The publishers no doubt have done their market research and are exploiting the present vogue in natural history and the oceans. Sales will go up before Christmas, since such books will catch the eye of parents seeking presents; these books may get into school and public libraries, and individuals may indulge themselves. As a marine biologist, I like *The Undersea* best. All the books, however, contain an excellent range of colour photographs, and even if the text of some of these books is banal in places they are a pictorial delight.

J. H. S. Blaxter

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## Medicinal and herbal plants

PUBLISHERS seem to be producing an army of books on the power of plants—how to grow them and use their products. Here are two more popular texts for those inclined to do-it-yourself medication or those interested in the subject from a more academic angle.

*Guide to Medicinal Plants* by P. Schauenberg and F. Paris (Lutterworth: Guildford, UK, 1977; £5.95) is translated from the French and is a straightforward, workman-like production. It is responsible in its attitude towards a subject which is not always treated with the respect it deserves: there may be a thin line between therapeutic and poisonous doses in some medicines of plant origin.

The plants are arranged according to their active principles—alkaloids, flavonoids, saponosides, tannins, and so on—each chapter being prefaced by a short

compresses makes fascinating reading: a gargle based on sage and red wine might make a sore throat almost worthwhile.

The occasional line drawings scattered through the text are not very helpful and could well have been omitted, but the coloured drawings at the end, though not outstanding, are pleasant and the plants recognisable.

*A History of Herbal Plants* by R. Le Strange (Angus and Robertson: Lewes, UK, 1977; £8) is in complete contrast. The author has obviously read widely, if somewhat indiscriminately, and has, it seems, put down as much about each plant as his publisher would allow. Unfortunately, not all the information is accurate, and the nomenclature, both Latin and English, leaves something to be

desired—the Oregon grape has been known as *Mahonia aquifolium* for very many years now—but this no doubt is due to inadequate sources of reference.

Each entry is illustrated but the drawings are often rather odd and in a number of styles; some, such as that of *Viola tricolor*, resemble those in Victorian gardening books and are charming, but some of the others are formalised to the extent of looking like decorative tiles, as in the *Aristolochia clematitis*. *A History of Herbal Plants* is, however, quite entertaining.

Rosemary Angel

Rosemary Angel is Head of the Museums Department at the Royal Botanic Gardens, Kew, UK.

## Making use of twigs

*Plants with a Purpose*. By Richard Mabey. Pp. 176. (Collins: London, 1977.) £4.50.

RICHARD MABEY wants us to recapture the sense of intimacy with plants that was known to our ancestors, and to help us he has written a book about the household uses of some of the commoner wild plants of Europe and North America. The result is a hybrid between a historical survey and a collection of helpful hints, which at first glance might seem appropriately subtitled *A Hundred and One Things to do with Twigs*.

The versatile twig and its close relatives, the stick and the sprig, certainly feature prominently, and it is possible to imagine a culture based on their use. In the dark ages, before science and technology began, the twig people would rise early from their heather mattresses, and chew on twigs to clean their teeth. They used bundles of birchwood to light their fires, on which they cooked a mess of pottage prepared using whisks made from handfuls of birch twigs—very satisfying to use, though rather fiddlesome to wash, Mabey assures us. They swept

their floors with birch brooms and strewed them with pine twigs. Each day would include an expedition to the forest to collect twigs. Older twig people took their ash walking sticks, and everybody wore sprigs of elder or wormwood in their hats to keep flies at bay. The day's collection of woody material was carried home in wicker baskets woven from willow twigs. When a woodland fire was encountered, it was beaten out using a bunch of fine birch twigs—presumably larger than those used for the whisks. The twig children usually played with catapults made from small forked sticks.

It seems unlikely that this book will stimulate a renaissance of the twig culture, or start a mass vogue for rush-lighting, nut polishes or homemade herb shampoos. Doubtless, the Forestry Commission would not be overjoyed if it did. But a *pot pourri* or a pressed-leaf bookmark is a simple reminder of the versatility of plants. Richard Mabey's book includes many such reminders, and will encourage a greater appreciation of the value of plants. Perhaps it will even help to restore their former intimacy.

Mary Lindley

Mary Lindley is Assistant Editor of *Nature* and a former student of botany.

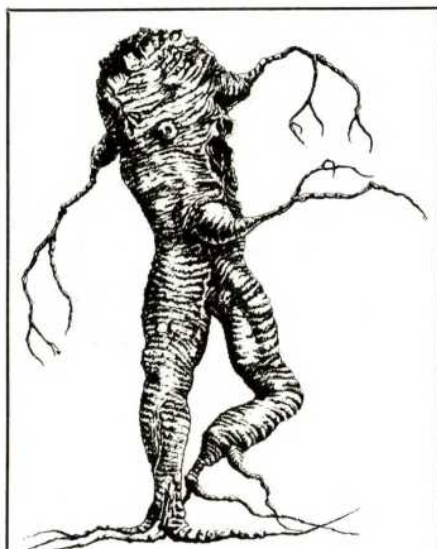


Illustration of *Bryonia dioica* (root), taken from *A History of Herbal Plants*, reviewed on this page. In the fifteenth and sixteenth centuries, the roots of the *Bryonia* were roughly shaped to represent the human form and implanted with Millet sprouts, to simulate the hairy Mandrake. These were then sold for large sums of money because of their reputed medicinal powers.

account of the constituents under consideration. Most of the plants listed are either native to or commonly cultivated in Europe, and at the end of the book there is a chapter on medicinal plants from other continents. This latter seems to be a fairly arbitrary selection, varying from coffee and sarsaparilla to nux-vomica. The entry for each plant is short but adequate, giving Latin, French, German and English names, distribution, a short description, flowering and collecting seasons, active constituents, properties, and so on. A chapter on tisanes and

## Victorian Natural History

*The Family Naturalist*. By Michael Chinery. Pp. 192 (Macdonald and Jane's: London, 1977.) £6.95.

I WAS fortunate enough to have a mother who was born in 1873, when Queen Victoria was on the throne, and who was one of the earliest women students of biology at what was then the Durham College of Science in Newcastle upon Tyne. Marriage prevented her from exercising her pro-

fession, but she introduced me to Natural History. From the age of three, we collected wild flowers, probably illegally, in the untidier parts of the Pollock Estate in suburban Glasgow. When, at the age of seven, I moved to rural Renfrewshire, I could already identify many of the common plants and insects. I pressed flowers and reared caterpillars in the nursery, and scared the maids with newts which escaped and found their way into the kitchen. We had all the texts for the well-brought up child. I remember particularly *Eyes and No Eyes*; this and other books of the same genre deserve to be reprinted.



This interest in Natural History was not as elitist as some may think today. My earliest mentor, outside the family, was David Borland, one of the few surviving handloom weavers in the village of Kilbarchan. He was a rugged independant, enjoying a penurious self-employment which gave him freedom to take time off and wander the countryside with two or three boys, who were constantly fascinated by his knowledge of wildlife and his powers of observation. I continued to be lucky when I went away to school, as on half-holidays I was allowed to explore the Teesdale countryside, occasionally guided by Bentley Beetham, who later graduated to Everest expeditions, from climbing cliffs to photograph peregrines.

Children today are not so fortunate. They do indeed have a marvellous selection of glossy Natural History books, lavishly illustrated, but few of these contain the sort of "meat" so satisfying to the enquiring child, that the improving Victorian writers and teachers provided. Michael Chinnery's *The Family Naturalist* fills this gap. It explains how to record, to observe and to investigate all aspects of Natural History. It is written simply enough to be understood by quite young children—at seven, I would have made it my bible. Even parents with no previous knowledge of the subject will be able to follow the text and the instructions. At the same time, professional ecologists who find it difficult to communicate their interests to their own children will find it invaluable—it may even enlighten them on some aspects of their own speciality. Although Michael Chinnery adopts the approach of the best Victorian naturalists, he is also uncompromisingly contemporary. He realises the need for conservation and the danger the ardent collecting habits of the nineteenth century naturalists would present if they were adopted by thousands of present-day children, when pressures on our flora and fauna are so much greater.

The book is therefore warmly recommended to both parents and children, whether or not they are already actively involved with Nature. Even town dwellers who only visit the country occasionally will find how to make those visits more interesting, and learn what (and what not) to take home for further study. Some of the exercises can even be carried out in urban areas.

Even this excellent book, however, will not work miracles. Some parents will be disappointed if they find they have children with no in-born interest in Natural History. My elder brother, brought up in the same environment as I was, never became remotely interested in plants or animals. But there are a great many parents and children to whom Michael Chinnery's splendid book will bring great pleasure and a much better understanding of the world in which they live.

Kenneth Mellanby

Kenneth Mellanby has been Director of Monks Moor Experimental Station, Abbots Ripton, UK.

## A talent to amuse

*Worlds within Worlds: A Journey into the Unknown.* By M. Marten, J. Chesterman, J. May and J. Trux. Pp. 208. (Secker and Warburg: London, 1977.) Hardback £7.95; paperback £3.95.

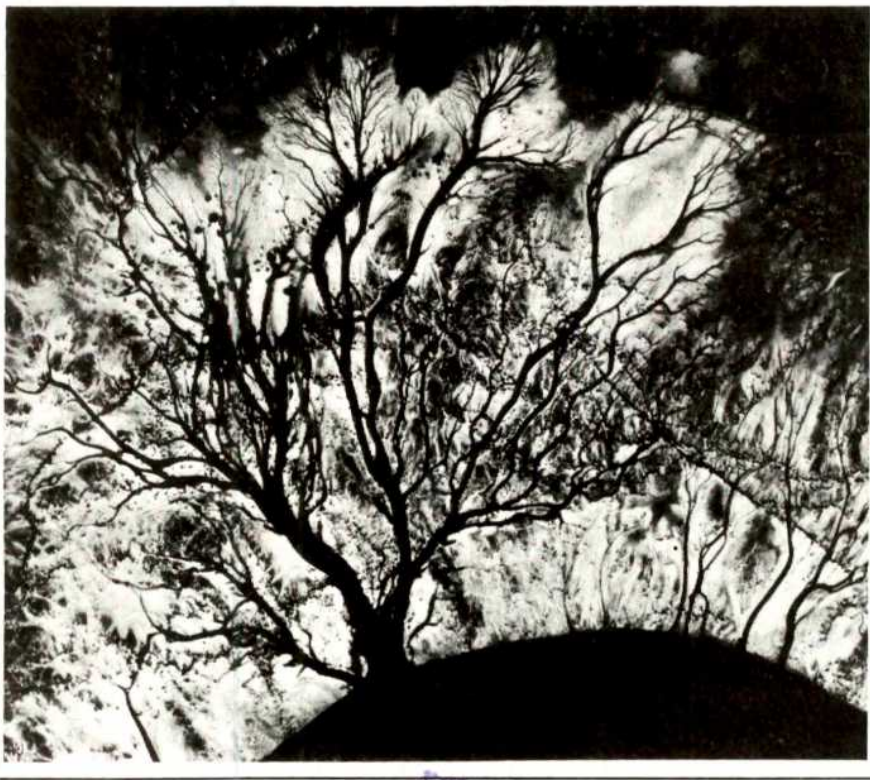
PERHAPS the least effective method of obtaining funds for a project is to argue that the results will provide pleasure for idle people. But when more telling arguments have extracted the money, the technology has been developed, and the results have appeared, then the pleasure may be there all the same and we would be foolish not to take advantage. That we can do with *Worlds within Worlds*, a collection of pictures resulting from advanced technology that allows us to be entertained during moments of idleness.

Not all such collections have the ability to entertain. The authors of this one are therefore to be congratulated on their choice of material. In a book of some 200 pages, they present a sample of images covering the wavelength range from audio to X rays. It has been done before—indeed, it has frequently been overdone, and certain topics have appeared with a sickly regularity—but *Worlds within Worlds* provides a refreshing change. The

authors present an intelligent selection of pictures, including many old favourites: bullets bursting balloons, diffraction patterns, crystal structures, marine organisms, bacteria, and images of our internal organs. Satellite cameras produce fascinating views of the Earth and its weather; and the Solar System is the source of a variety of stunning photographs. But the most fascinating photographs are of the effects produced by invisible objects—heat images. False-colour images from computer coding provide a contrast with the realism of optical wavelength photography; and the authors are sensible enough to include a judicious selection of black-and-white photographs. The result is a book of surprises and contrasts with great visual appeal.

The same intelligence can be detected at work in the figure captions, but with less success. It is unfortunately a luxury to expect information from the text of a book of this type; yet, here the pictures have been chosen not only for aesthetic reasons but also because there is a story to tell. Often the captions are interesting and informative, occasionally fascinating, but sometimes they degenerate into the naive and sensational. Although below the high standard of the pictures, the text nevertheless adds to the enjoyment of the book rather than being an irritant. Relating the text to the pictures, however, can be frustrating: faint numbers can be seen below the pictures

*Colorado River Delta in the Gulf of California. The River is the black expanse at the bottom of the picture, with its waters branching out through the white sandbars to gain access to the sea at the top. Illustration taken from Worlds within Worlds, reviewed on this page.*





after some hard searching, but they could usefully have been more obvious while remaining unobtrusive.

A brief guide to the electromagnetic spectrum, and the techniques used to explore phenomena at different wavelengths, rounds off the book. This is not a reference book and not a book

to be read, but it is more than a coffee-table book designed to impress. An intelligent choice of pictures and words makes this a volume that will fascinate and inspire. It is well worth the asking price.

**Stuart Sharrock**

*Stuart Sharrock is Physical Sciences Editor of Nature.*

## Palaeontological pop

THE Christmas dinosaur season is upon us once again. Two major offerings of large format by Richard Moody and Michael Tweedie both have authoritative texts. Moody's *Natural History of Dinosaurs* (Hamlyn: London; £2.95) has a 29-page introduction that is packed with information, highly condensed and is by no means an easy read. Evolutionary changes are documented but for my money I would have liked to have seen a more extended discussion of the functional aspects, and at least something on the other aspects of the contemporary faunas to give a more rounded picture of the natural history of dinosaurs. There are one or two jarring bits of information. I have never heard of the pelycosaur *Dimetrodon* having its teeth "differentiated into incisors, canines and cheek teeth" and the occasional purple passage such as certain dinosaurs "grinding mouthfuls of 'Mesozoic cud'" have implications with regard to feeding habits that are unfounded. But really I have no quarrel with the author's contribution. It is a worthwhile job well done, given the limited space at his disposal.

The main part of Moody's book is a series of plates with explanatory accompanying texts. The type-face is larger, and the mini-articles are easy to read and surprisingly informative; again the author has done a good job. This book is clearly intended to sell as a picture book, and five different artists have contributed to this. Ann Baum is a most accomplished artist and her illustration of *Acanthopholis* playing wheelbarrows is far and away my favourite dinosaur illustration. It is a charming picture and leaves one with the impression that the range of dinosaur behaviour was more varied than we had previously imagined. My next favourite is Tony Morris's duckbilled dinosaur *Brachylophosaurus* shown in a tender embrace of what could only have been the last waltz. A few illustrations are indescribably bad and grossly inaccurate. Finally it's a great shame that there were only two illustrations by Thomas Crosby-Smith. His *Mandasuchus* feeding on the corpse of a dicynodont in the pouring rain is magnificent. In conclusion, as a picture book, this is very much a cōfite's egg (good in parts) and I feel that the excellence of the text has not been adequately matched by the artwork.

*The World of Dinosaurs* (Weidenfeld and Nicolson: London; Morrow; New York; £4.95; \$14.95) by Michael Tweedie is clearly the work of an extremely accomplished writer. It is easy to read and gets across a considerable amount of information. Yet, on reading it, one gets a feeling of *déjà vu*. I suppose Alan Charig and friends should be flattered, but it is clear, at least to my colleagues, that this book is obviously derivative. One should not carp about this too much, for it is done with flair and in itself is an excellent account of dinosaurs. The running text is well illustrated, but interspersed at intervals are large coloured double-page spreads, a few are grossly inaccurate—for example, the ceratopsians the sauropods and the marine short-necked plesiosaurs. Other illustrations have a familiar ring and I am reminded of Giovanni Caselli proudly claiming that other artists would henceforth use his work as reference material; I am certain he will be suitably gratified to find his prophecy fulfilled.

The last two books are on fossils in general, and in the main are on the kind that anyone can hope to find. Rhona Black's *The Observer's Book of Fossils* (Frederick Warne: London; £1.10) is a neat pocket-sized book of modest price, which, after a concise introduction dealing with many aspects of palaeontology, gives a systematic run through of the major groups of fossils with paragraphs on the commoner forms. The line illustrations are perfectly adequate, al-

though their positioning at the bottom of each page gives the impression that they were an afterthought popped in just to fill up the page. The complete absence of an index is infuriating and must reduce the utility of this book enormously. The only other criticism I have is in the section on fossil collecting. My own preference is that notebook and pencil and wrapping paper are the prime requirements. So much unnecessary damage is caused at fossil localities by the over-enthusiastic use of hammers and chisels that their use should be positively discouraged; they are hardly ever necessary and do more harm than good. If fossils cannot be collected by hand, they should be left where they are.

With the final book on fossils, we are back to the Christmas market. Richard Moody's *The Fossil World* (Hamlyn: London; £2.95) is a glossy picture book with attractive colour photographs of fossils. Instead of trundling through one fossil group after another, Moody has dealt with the three major fossiliferous eras: the Palaeozoic (43pp), the Mesozoic (23pp) and the Caenozoic (17pp). The text is of a high standard and reading through it one gains a vivid impression of the changing fortunes of animal life through geological time. Again, I would have preferred rather more on the functioning and behaviour of the organisms he describes. Lots of technical terms suddenly pop up without any explanation and I personally found the sections dealing with bivalves and echinoderms particularly hard going. It was a surprise that the coccoliths as the major component of the Chalk rated not a mention in the Mesozoic section. Basically, I liked this book very much and I hope it does well.

**Beverly Halstead**

*L. B. Halstead is Reader in Geology and Zoology at the University of Reading, UK.*

## Megalithic architecture

*The Megalith Builders.* By Euan MacKie. Pp. 208. (Phaidon: Oxford, 1977.) Paperback £4.95.

THIS is a popular book, without references in the text either to sources or to the numerous photographic illustrations, which are of varying quality. The colour-plate of Stonehenge (p17) and the aerial photo (p77) and the interior colour view (p88) of the West Kennet Long Barrow are laterally reversed. The colour plate on p190 and the cover, ostensibly of Stonehenge near sunset in winter, is a clever photomontage, visually attractive but astronomically impossible, and should have been identified as such. Several of the black-and-white plates are dark, muddy or out of focus.

The author's declared aim is to "explain" the megalithic monuments of western Europe in their current chronological contexts. Formerly, it was supposed that megalithic architecture was diffused gradually from the Mediterranean around the western and northern coasts of Europe; but latterly corrected radiocarbon dates (and a series of thermoluminescent dates from Portugal with wide confidence limits, to be used with more caution than the author shows) have tended to imply that the earliest tombs were built on the Atlantic littoral. This is inconsistent with the older model of unidirectional diffusion from Mediterranean or Near Eastern sources of cultural innovation.

The scene is set by eight rather disjointed chapters on regional groups of sites. A surprising inclusion at the beginning is Skara Brae in Orkney, a



block of late Neolithic one-room flatlets hardly to be described as megalithic. An even more surprising omission is any treatment of the abounding megalithic tombs of the Netherlands, north Germany and southern Scandinavia. Thus, the descriptive foundation of the book is at best partial. It also contains avoidable errors.

The interpretation begins in chapter nine with an interesting examination of the nature and limitations of explanation in prehistory, which deserves close but critical attention. Mackie rightly says that "it is impossible to proceed directly from the archaeological evidence to the detailed reconstruction of the vanished societies which produced it"; but he believes that social explanations can be provided "by analogy—by looking at the known primitive peoples who possess a material culture similar to the prehistoric one" (and what about a similar environment?). There follows an imaginative discussion of alternative mechanisms of cultural change, modelled by analogy on theories of biological evolution. The Lamarkian, anti-diffusionist, model allows cultural or technological innovation to arise spontaneously (and maybe more than once in different times and places) in response to the stimuli of local conditions, natural and human. By contrast, the Darwinian model requires that an initial cultural mutation spreads ever wider from its source by a process of successive out-breeding and hybridisation.

On this basis, one would have thought that Gordon Childe, the chief architect of the diffusionist hypothesis in European prehistory, would have been classed as a neo-Darwinian. Surprisingly, however, MacKie sees him as a cultural Lamarkian, quoting these words from Childe's *Social Evolution*: "Inventions can be transmitted from one society to another, and that is precisely what diffusion means. But that is just what is impossible in organic evolution".

When this was written in 1951, it was believed to be true; but subsequent advances in molecular biology have shown that innovations can be transferred from one group of micro-organisms to another by genetic recombination. In this sense Childe's model of cultural diffusion was even more closely neo-Darwinian than he himself could have admitted. MacKie's analogy here is all the more strange, in view of his firm support for the model of social evolution put forward by C. D. Darlington in *The Evolution of Man and Society* (1968), in which cultural and genetic out-breeding reinforce each other through a synergistic

hybrid vigour, with the corollary that in both fields continued in-breeding leads to stagnation, etiolation and ultimate extinction.

The rest of the book is an attempt to explain the long and diverse development of European megaliths in Darlingtonian terms, as the result of the cultural and genetic impact on various neolithic societies of a class of far-voyaging astronomer-priests, itself the product of the expansive hybrid vigour of the early urban societies of the Near East. Here once more are Childe's "megalithic saints", though more exotically attired.

This is an interesting thesis, but its foundations are dangerously insecure. "That professional priesthoods existed in north-west Europe in the middle of the third millennium B.C. . . .", says MacKie, "is abundantly testified to by the stone circles and the inhabited earthworks and stone villages of late Neolithic Britain". But this is not testimony. The evidence itself compels no such conclusion, nor indeed any specific conclusion, because it is material, text-free and dumb. It has to be questioned, and the questioner supplies the answers himself. It is all too easy (and that is why the temptation should be resisted) to mistake an attractive but arbitrary speculation for an inference which the evidence itself imposes.

## Amateur star-gazing

THE pace of astronomical and space research, the speed with which technological developments in optical and radio astronomy are implemented, the inventiveness of the astronomical mind and the productivity of the astronomy book publishers never cease to amaze me. This year, like others, the bookshops bulge with new titles and reprints of old favourites all eagerly awaiting buyers of all ages. This review considers ten books that are ready to be snatched up by the present-seeker as the festive season nears.

Let us start in space. *Flight to Mercury* (Columbia University: New York and Guildford, UK; \$16.20) is a diary of the Mariner 10 mission to Venus and Mercury, compiled by Bruce Murray, the director of the Jet Propulsion Laboratory of the Californian Institute of Technology and Eric Burgess, the well-known author of many technical and popular articles on upper atmospheric physics, rockets, missiles and space flight. Mariner 10 was launched in November 1973. Because of the economic recession at that time, this mission set new standards of cost control and efficiency. It was Man's first detailed look at Mercury, the innermost planet.

Although it is true that much archaeological explanation does rest on analogy (the author rightly says that we call a particular bronze artefact a spearhead because it resembles artefacts used as spearheads today), the ambiguity of identification by analogy increases very rapidly as we move away from technology to social structure and religion. In these fields analogy can explain little or nothing about the prehistoric past. It can do no more than enlarge the field of conjecture.

Furthermore, it is worth asking whether much of the "megalithic problem" is not really a non-problem. By definition, a megalithic tomb or temple is built of large stones. It has been assumed for centuries, and still is, that most if not all of the prehistoric megalithic constructions of Europe are manifestations, however diverse, of a common basic phenomenon, and require a unifying hypothesis to explain them. But why? No-one seeks a corresponding single hypothesis to explain the distribution, diversity and chronology of contemporary constructions of small stones, or for that matter of timber. May we not be searching in the dark for the black cat that isn't there? **R. J. C. Atkinson**

*R. J. C. Atkinson is Professor of Archaeology at University College, Cardiff, UK.*

It produced thousands of striking photographs of Mercury and Venus, over 100 of which are beautifully reproduced in this book. The book is riveting; reading it, you feel as if you are there, at mission control, living through the problems and triumphs with the members of the science and engineering teams. I didn't realise before just how many things go wrong with "successful" missions: television heaters don't come on, antenna power unexplainedly drops, manoeuvring gas nearly runs out, instrument bays heat up, bogus satellites of Mercury are discovered. The book is a space-fact adventure story and I recommend it highly.

From space fact to space fiction. *Colonies in Space* (Stackpole: Harrisburg, Pennsylvania; Van Nostrand Reinhold: Wokingham, UK; £9.85) by T. A. Heppenheimer, is just that (at the present time); but who is going to be dogmatic enough to say it could not happen. Heppenheimer shoots us forward to the days of space colonisation, tens of thousands of people living in attractive Earth-like space communities. We read of space farms, closed-cycle ecosystems, Moon Miners, asteroid tugs, orbiting power satellites, low gravity swimming pools, and interstellar flight. We are shown beautiful paintings of the interior of a space colony, most of these from the

brush of Donald E. Davis, a NASA artist. Heppenheimer is a planetary scientist, and in this well written book he draws on many serious and careful studies that show that Man has the scientific and engineering capability to put human life permanently into space. In his words, this "is no less than a major new stage in human evolution, the first step to star trekking across the light years of our universe". Heppenheimer paints a Eutopia in the sky but seems rather shy of counting the cost to those left behind on Earth. I'm left with the sneaking feeling that it would be easier to make a 'heaven on Earth' first.

For the Earthbound telescopicist, *Moon, Mars and Venus, A Concise Guide in Colour* (Hamlyn: London; £1.50) by Antonin Rükl, is an excellent buy. It contains a brief summary of Man's exploration of the lunar near side, a discussion of the height and formation of lunar features, the views of Venus and Mars that can be seen through a telescope, and a short history of the observation of these planets from Earth and using space probes. The bulk of the book is taken up by a series of small (about 10° by 14°) detailed maps of the nearside of the Moon. These are complimented by notes on the opposite page giving details of crater sizes and depths, and the occupation and country of origin of the dignitary the crater is named after. There is a ten-page section of Mars maps. The word colour in the title is rather misleading as all the maps are just brown with a blue border, they could equally well all have been black-and-white. Also, as the book is obviously geared to the observer with a small telescope, I think it would have been more useful to have the maps in 'telescope' coordinates with North at the bottom and East on the left. As it is, the book shows the Moon as it appears to the naked eye, with normal selenographic upright coordinates. These are, however, minor quibbles with what is clearly a well written, beautifully drawn, inexpensive book, a book which is bound to become a firm favourite with amateur astronomers.

I have before me four books by Patrick Moore, the genial presenter of the British Broadcasting Corporation's television series *Sky at Night*, which has continued monthly without a break since April 1957. As a populariser of astronomy and space science, Patrick Moore is without equal. His prolific pen produces books for the beginner in astronomy, books which read easily; in fact, books which read exactly as Moore speaks, with staccato, bubbling, infectious enthusiasm. *The Story of Astronomy* (Macdonald and Jane's: London; £5.95) was first published in 1961 and is now in its fifth, revised edition, having been brought completely up-to-date in both text and illustrations. It is an achievement for any

book to get to five editions; and reading this one, one can easily understand why it did. It is an ideal introduction to astronomy for the young reader; in fact, one could go so far as to say that every bright youngster should have a copy. It is clearly, and in many cases beautifully, illustrated. Moore starts with stone-age observations and tells the story of the stars, planets and galaxies right through to the time of the Viking landing on Mars.

*Guide to the Comets* (Lutterworth: Guildford, UK; £4.25) is a revised edition of Moore's 1973 book. It contains descriptions of the basic observed structure of comets, how they fit into the Solar System family, and how they are discovered by the enthusiastic band of comet hunters. Halley's comet and Encke's comet are discussed in detail, and the book ends with a discussion of lost comets and theories of cometary origins. Sixteen photographs are included, and the book has useful lists of known periodic comets. So far as it goes, the book is a good introduction but I can't help feeling that it would be greatly improved if it went slightly further. A 25% addition to the 96 pages could have touched on such topics as the way orbital parameters vary between cometary families, recent space-lab observations of cometary spectra, comet tail formation, comet decay and meteor stream formation, factors affecting the choice of comet nuclei models, and cometary outbursts.

Patrick turns his gaze on to the Red Planet in *Guide to Mars* (Lutterworth: Guildford, UK; £5.95). Again, the subject is approached historically, starting with the pre-1830 division of the Martian surface into dark areas and white polar caps, moving through the canal controversy of Schiaparelli and Lowell to the modern items of photography from Mariner Space probes and surface details, and the excitement of the search for life from the Viking craft. Mars has long been a world of conjecture; the canals have vanished into the realm of science fiction but the problem of life or no life is still very much with us. (Harping back to Heppenheimer, even Martian colonies are not beyond the bounds of possibility.) This book is a mine of information, and has many maps and photographs; I recommend it to anyone who wants a relatively painless introduction to the intricacies of this planet.

*The Astronomy of Southern Africa* (Robert Hale: London; £4.95) by Patrick Moore and Peter Collins, traces the history of South African astronomy from 1658 when Father Guy Tachard, a Jesuit priest en route for Siam, set up an observatory in Cape Town, to the present day when the country is liberally dotted with visual and radio observatories. Moore and Collins discuss the work of L'abbe de la Caille, who charted the positions of almost 10,000 southern stars

between 1751 and 1752, Sir John Herschel who set up a great telescope of 20-feet focal length in 1834, and Sir David Gill, one of the most outstanding of all the astronomers of South Africa, and many others. They also sketch the histories of the Royal Observatory Cape of Good Hope, the new Sutherland Observatory, the Government Meteorological Observatory and Union Observatory, Johannesburg, the Leiden Station, Hartbeespoort, the Yale Observatory and the Radcliffe, Pretoria. The illustrations (many from museums and archives), the many glimpses into the lives of the observers and the sensible discussions of their aims and achievements, make this book a joy.

While south of the equator, let us briefly discuss *Astronomy for the Southern Hemisphere* (Reed: Wellington, Sydney; Bailey: Folkestone, UK; £10.85) by Lionel Warner, which caters especially for star gazers in those parts. Mr Warner is obviously a very successful teacher, and his descriptions of some of the demonstrations that he uses in his classes come over very well. It is an excellent first book for the astronomical 'doer' as opposed to the astronomical reader. I found his monthly star maps, however, much too barren.

J. Hedley Robinson's *Astronomy Data Book* (Wiley: New York; £8.25; \$13.90) is rather like the curate's egg, only good in parts. I was very impressed by some of the tables listing, for example: brightest galaxies; variables observable with the naked eye over all and part of their brightness ranges; specific variable types, such as T Tauri, T Orionis, R Coronae and U Geminorum; binary stars; and the 286 brightest stars, all of which will be most useful to the amateur observer. Other tables such as, for example, a simple alphabetical list of formations on the far side of the Moon were much less useful. The glossary, with its very sharp, few word entries, I found to be distinctly unhelpful and in certain cases misleading.

Finally, we come to *The New Astronomy and Space Science Reader* (Freeman: San Francisco and Reading; hardback £12/\$15; paperback £5.50/\$7.50) edited by John C. Brandt and Stephen P. Maran. This book contains a collection of some 44 articles on current topics in optical, radio and space astronomy research both observational and theoretical, many of which are only briefly touched on in formal textbooks. These articles have been gleaned from such journals as *Natural History*, *Smithsonian*, *Scientific American*, *Science*, *Nature* and the *Astrophysical Journal*. The book provides a superb bridge between textbooks and the immense and rather frightening world of the professional journal. All amateur and student astronomers will gain immensely by reading it.

David W. Hughes

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# obituary

## Jacques Tréfouël

PROFESSOR Jacques Tréfouël, Director-General of the Pasteur Institute in Paris from 1940–1965 and a figure of great influence on the French scientific scene during this period and for many years after his retirement, died on 11 July 1977 at the age of 80.

He was an outstandingly capable and skilful organic-synthetic chemist who dedicated his whole active scientific life to the field of chemotherapy. He entered the Pasteur Institute at the age of 23 when he became a student of the great master of French chemotherapy, Ernest Fourneau, head of the Department of Chemotherapy created by the then Director-General of the Pasteur Institute, Emile Roux. A year later he married a colleague of his, Thérèse Boyer, who was not only an equally brilliant synthetic organic chemist, but also an exceptionally attractive lady of great charm and elegance. Nearly all his original papers were published jointly with his wife, and Jacques and Thérèse Tréfouël became one of the most productive and best known scientific couples. They stayed at the Pasteur Institute until their statutory retirement.

Their first papers, most of them published jointly with E. Fourneau, followed Paul Ehrlich's lines and led to a series of substances active against trypanosomes, spirochetes and plasmodia.

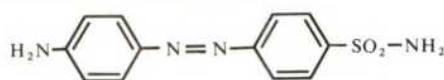
The most important contribution of the Tréfouëls made in collaboration with their brilliant colleagues the pharmacologist Daniel Bovet and the microbiologist Frederico Nitti, which earned them a position of immortality in the biomedical sciences, was the discovery in 1939 of the chemotherapeutic properties of sulphanilamide against bacterial infections.

During his extended histological studies, at the end of the last and the beginning of this century, Paul Ehrlich had become aware of the strong antibacterial properties of some of the synthetic aniline dyes, but unfortunately all of these were far too toxic for systemic chemotherapeutic use. However, Paul Ehrlich hoped, in view of the fact that dyes were capable of specifically staining some part of cells while leaving others unstained, and in view of his firm belief that the possibilities of synthetic organic chemistry were practically unlimited, that one day a synthetic dye would be discovered



which would specifically kill bacteria without affecting the host organism they had infected. He was encouraged in these thoughts by his finding that the dyes trypan blue and trypan red, were moderately effective in the chemotherapy of trypanosomal infections.

Stimulated by Paul Ehrlich's ideas the pharmaceutical divisions of the German dyestuff industries, then leading the world, made a tremendous effort to test thousands of dyes for chemotherapeutic efficiency in bacterial infection, but without success. In 1935 G. Domagk, of Bayer's, reported the sensational news that finally he had found an azodye, prontosil



which was capable of protecting mice against streptococcal infections and was also effective in man. This dye had been tested for over two years before its chemotherapeutic properties were released for publication. It thus seemed that Paul Ehrlich's ideas, after a period of over 30 years, were finally vindicated. However, the Tréfouëls, Bovet and Nitti proved conclusively a few months later in the same year that the chemotherapeutic properties in bacterial infections of prontosil had nothing to do with its dye nature, but resided totally in the non-coloured moiety sulphanilamide of the prontosil molecule. They postulated that prontosil was reduced to sulphanilamide in the animal body, an hypothesis which was later proved correct experimentally and explained why prontosil was inactive *in vitro*, but active *in vivo*.

There can be no doubt that the discovery of the chemotherapeutic

properties of sulphanilamide marked a major revolution in therapeutic medicine, both with regard to its practical use and theoretically. Sulphanilamide, synthesised as early as 1908, is a very simple molecule and can be readily modified. The Tréfouëls synthesised over a hundred derivatives, among them the sulphones which later were recognised to be valuable chemotherapeutic agents in the treatment of leprosy. Since then many thousands of sulphonamides have been synthesised in the laboratories of numerous pharmaceutical firms, and the advent of the antibiotics has not eclipsed them. They are still widely used in the clinic, particularly for the treatment of urinary infections caused by *E. coli* in combination with folic acid antagonists of 2,4-diamino pyrimidine structure (sulphanilamide itself was shown in 1940 by D. D. Woods in this country to antagonise the structurally similar para-amino benzoic acid, a part of the folic acid molecule, a discovery which, on its own, signified a major advance in the science of biochemistry).

Professor Tréfouëls scientific achievements were rapidly recognised and rewarded inside and outside the Pasteur Institute. He was promoted to Head of the Laboratory and, a few years later, to be Head of the Division of Chemotherapy. In 1940 he reached the administrative pinnacle of the Pasteur Institute when he was nominated Director-General. He was put into this prominent position while France was passing through some of the most difficult years of its history.

In 1939 World War II broke out and a year later France was conquered and humiliated by Germany. Jacques Tréfouël, thanks to his inexhaustible spirit of enterprise, his great diplomatic talent and flexibility, his courage and his firm, deeply felt and uncompromising patriotism, managed to steer the Pasteur Institute successfully through very stormy waters with dignity and determination, and to preserve the essence of its unique historic scientific traditions and patrimony. He conceded nothing of importance of the possessions of the Pasteur Institute to the enemy, and his authority inside the Institute remained untarnished and undisputed.

He was a very courteous and polished personality, unruffled in situations of difficulty, and his radiant smile was irresistible. He was very erudite and wise, and was always very



kind and friendly, though he could be quite firm in relation to the people with whom he had to deal. He was gifted with a quick intelligence which enabled him to understand complex situations in a few moments, and he was endowed with a very refined, inimitable and typically French sense of humour.

His exceptional human qualities enabled him to keep on good terms with the majority of his senior colleagues and they made him popular among the staff of the Pasteur Institute. He appointed judiciously and, on the whole, successfully, new members of the staff and succeeded in maintaining the very high scientific standards of the Institute. He also created and maintained close contacts with the French pharmaceutical industry, to both its own benefit and that of the Pasteur Institute.

In my view, the Pasteur Institute never had a more dedicated and successful director than Jacques Tréfouël.

Throughout his onerous social duties as Director-General of the Pasteur Institute, Jacques Tréfouël received extensive, continuous and competent support from his wife who combined the properties of an excellent scientist with those of a perfect hostess. Time passed quickly during the animated and witty conversations covering a wide range of subjects at their elegant parties, which remained an unforgettable experience in the memory of those privileged to participate in them.

Jacques Tréfouël received numerous high honours from the French and other Governments and scientific societies. Among these were his nomination to the grade of a Grand Officer of the Legion d'Honneur, to the Membership and later Presidency of the French Academy of Sciences and the National Academy of Medical Sciences, many honorary degrees in the Universities of Europe, including Oxford and Cambridge in this country, and the Americas, and membership of many foreign academies and learned societies.

The passing of Jacques Tréfouël marks the end of an era in European science.

*Ernst Chain*

## G. K. Green

GEORGE KENNETH GREEN, or Ken Green as he was known in the world of particle accelerators, died on 15 August 1977 of a heart attack while visiting his son at Brownsville, Texas. He was 66 years of age and had lived through, and taken a leading part in, one of the most extraordinary technological de-

velopments of our time.

Ken Green was born in St David, Illinois, and retained all his life the looks and the dry humour of a Mid-Westerner. He went to Illinois University, obtained a Ph.D. degree in physics there in 1937, and stayed on a year afterwards as an Associate in Physics. His first experience with what became his life's work was at the Radiation Laboratory of the University of California where he did design work on cyclotrons and nuclear physics research. It was there, at what was then the fountainhead of accelerator developments, under the inspiration and leadership of Ernest Lawrence, that Ken Green must have fallen in love with accelerators. He also spent a short time at the Department of Terrestrial Magnetism of the Carnegie Institution of Washington D.C.

He spent the war years with the Army Signal Corps, joining in 1942 as a second lieutenant, and in 1946 he was Army Electronic Representative and Technical Head of the Signal Corps Group at Operation Crossroads at Bikini. Later, he became principal physicist at the Evans Signal Laboratory of the Signal Corps at Belmar, New Jersey. During this period he worked on sonar and was one of the inventors of the proximity fuse, an important advance in firing rockets and artillery. At the Bikini test of atomic bombs he was involved in the development of instrumentation to study nuclear explosions. For his services during the war he received both the Civilian Distinguished Service Award and the US Army Legion of Merit.

Ken Green joined the Brookhaven National Laboratory in 1947 to work on the 'Cosmotron' and he never left that laboratory nor accelerator building for the rest of his life. The Cosmotron, in its time, was the largest accelerator in the world, and with a top energy of 3 GeV it was the first accelerator to exceed one thousand million electron volt particle energy. Ken Green was involved in the design and construction of every part of this machine. Formally, he was a senior scientist; in practice he combined a considerable design ability across the whole field of accelerator technology with a remarkable talent for supervising the construction of this giant machine—a talent rather rare in those days. The result was that Ken Green knew every detail of the Cosmotron, the reasoning behind its design, how it was constructed, and how well or badly it operated. No wonder that he earned the name of 'Mr. Cosmotron.'

The construction of the Cosmotron was finished in 1952, and many im-

provements were made to it subsequently, in all of which Ken Green was intimately involved. Meanwhile, another, even bigger accelerator project was being quietly conceived at Brookhaven, and in Europe a new international laboratory was being set up, called CERN, which aimed at building a similar machine. Then followed one of those unofficial and extremely effective collaborations between two laboratories which prove so beneficial to the course of scientific research, and Ken Green entered whole-heartedly into this collaboration.

The two machines, the 30 GeV AGS at Brookhaven and the 25 GeV CPS at CERN were essentially designed together. The principle of alternating gradient focusing was discovered at Brookhaven at this time, and the two teams worked out the consequences of this important idea together, and based the design of their machines on this new principle. Brookhaven staff came to Europe, CERN staff went to Brookhaven, and the success of these two machines owed much to the close collaboration between Ken Green's team at Brookhaven and the CERN team at Geneva.

The two machines came into operation at about the same time in 1960, and the two teams celebrated each other's success. The AGS and CPS were for many years the highest energy accelerators in the world. Ken Green became the Chairman of the Accelerator Department of the Brookhaven National Laboratory in 1960, having first served for a while as deputy-chairman under Leyland Haworth, and he held that post until 1970.

His last most notable contribution to accelerator design was the electron machine for the National Synchrotron Light Source which he started before his official retirement from Brookhaven Laboratory and continued for the short while afterwards until his death. Construction of this machine is due to start at Brookhaven towards the end of 1977. His last appearance in Europe was in the spring of this year when he came over to CERN to participate in the inauguration of the European 400 GeV SPS machine.

Ken Green has earned an immortal place in the ranks of the great accelerator builders with the Cosmotron and the AGS which were not only the highest energy machines of their time but also very challenging projects, involving many technological advances. All his many friends and colleagues throughout the world, in all the laboratories where these giant machines exist, will mourn his death, and remember with gratitude the help and encouragement he has given them.

*J. B. Adams*

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## Engineering—big issues and little issues

THE Finniston Committee to enquire into the engineering profession is just about to get underway. The committee will be able to investigate pretty well whatever it wishes under the general headings of the needs of industry, education and training, the institutions, and registration and licensing. Many hope that this committee will be the first step along the road to affording engineers their rightful status, whatever that may mean, and that a more prestigious profession will attract and retain better talent, thereby contributing to a revival in British industry.

There is no doubt that at present the engineering profession is not a remarkably well-paid one, that its institutions are in a state of considerable disarray, and that it suffers from association with the perennially bad news that is put about concerning British industry and industrial relations. As a result of all this people are hardly falling over each other in an attempt to become engineers.

Take, for instance, the institutions. Undeniably an engineer is not created the moment he (or very occasionally she) emerges from higher education, and a period of development needs to be watched over by some form of peer review, for which the institutions serve admirably. And for a rather small number of engineers the institutions serve a continuing function of education. But to meet these needs there are 15 institutions, each with its own traditions, bureaucracy and fine distinctions (as to, for instance, what a technician is and whether a technician can be a member). And now there is another layer, the Council of Engineering Institutions, born in considerable travail and watched very carefully by the individual institutions. It would be easy for the Finniston Committee to spend much of its time sorting out these institutional problems of engineering; what is more, the institutions on the whole favour some form of registration and licensing of engineers (this is not done at the present) and the committee could equally get bogged down in trying to produce a blueprint that was acceptable to all parties—when it is not at all clear that registering, licensing and thereby more effectively disciplining engineers is anything other than a sideshow generating bureaucracy to relatively little purpose.

Instead, there are two very clear and major problems that the committee should face. The first is that of recruitment into engineering, meaning not the choice

made on graduating as to which company to join, but the choice made in school, maybe as early as 14 or 15, to opt for engineering as opposed to the natural sciences. Arguably this decision is forced on students too young, with most universities singularly unhelpful in allowing students to defer their choice until they have experienced a year or two of higher education. But whatever time it is made, it seems remarkable that anyone selects engineering at all in the present educational environment. Few teachers have any industrial experience, and if they have had such experience, as likely as not they have been discouraged enough by it to turn to teaching instead. Few schools have a decent and open relationship with local industry—and where there is no local industry (as in the rural environment of most public schools) contact with industrial life may be non-existent. Small wonder then that a life of engineering is seen by many young people as a poor alternative to a life of scientific research.

The second problem is that nobody really knows what the shape of British industry will be in ten or twenty years. In a completely cut-throat environment, many industries would shed vast numbers of workers, opting for much more automation at severely reduced manning levels, while other industries, such as shipbuilding, steel-making, and car manufacture might capsize because of foreign competition. In a more protective world, the government, aware of electoral implications, will fear to grasp any of these nettles but will prop up ailing industries and stand in the way of mass redundancies in the name of social policy.

In these circumstances it would be valuable to have a chart of possible paths for steering industry through to what is bound to be a totally different world by the year 2000. Economics and politics are bound to have major roles, but the engineering profession has a central part to play. Thus the Finniston Committee could provide a valuable service to the profession and to the nation by taking a longer-term view than governments ever seem to be able to take. It could also try to establish some general view of where industry will be by the end of the century. Some will carp that this is beyond the committee's remit, but if the needs of industry for engineers is one of the terms of reference then presumably the very character of that industry is rather germane to such discussions. □

# Against instant books

Stephen H. Schneider of the National Center for Atmospheric Research, Boulder, Colorado, explains why he feels that 'instant' books often do more to confuse than elucidate the scientific controversies they discuss.

TIME and again in recent years a chorus of social pundits has bemoaned the increasingly hectic pace of modern living. Among the shopping list of adverse side effects frequently cited are the break up of the cohesiveness of the family unit, the rise in feelings of societal alienation of many young people, the increase of environmental pollution, the rise of junk foods, and the alarming rate of stress-related disease. To this growing list we must add a contribution from the publishing industry: the 'instant book'.

For example, very soon after the harsh "Winter of 1977" in the United States, as it is often called, we have *The Weather Conspiracy: The Coming of the New Ice Age* (Ballantine, New York, 1977). It has many of the trappings of an instant book. Since its 'author' is "The Impact Team", a group of 18 non-weather experts calling themselves reporters, writers, researchers, and "back-up" (whatever that means) people, they had to turn elsewhere for scientific credibility. They chose the wrong people.

Space doesn't permit a detailed critique of the two CIA reports on climate, which are the basis for *The Weather Conspiracy* and are included as appendices, and upon which the book leans so heavily for what it calls "true facts". I must, however, mention that Professor Reid Bryson of the University of Wisconsin, whom the CIA and the Impact Team cite as the expert predicting most of the coming climatic disasters, has publicly repudiated much of the CIA reports; and they quote him as a principal source of specific climatic predictions. Bryson objected for the simple reason that the predictions were specific—something which is beyond the state-of-the-art skills of climatologists. In fact, much of the CIA reports depend on the pre-1974 views of Bryson, and he has himself argued that new evidence has required him, as any good scientist, to revise and recast his views. In essence, I would characterise parts of the CIA reports that predict the climatic future as "Early Bryson extrapolated", and much of *The Weather Conspiracy* thus as "Early CIA extrapolated".

I must, however, confess nagging conflicts that bother me in using *The Weather Conspiracy* as a butt:

- it includes an impressive amount of material on climate, even if there is little cohesive thinking to link it together; and I don't want to take the purist role and discourage all mass market attempts to "spread the word" about the very real dangers climatic issues do pose for society merely because such polarisations simplify complex issues;
- many of the Impact Team's proposed solutions to these dangers, that is, food reserves, weather control treaties, energy conservation, and so on, while not new to those who follow the issues, are plausible and need widespread exposure—something a mass market book can do well;
- most importantly, as one whose own book, *The Genesis Strategy: Climate and Global Survival* (Plenum, 1976 and Delta, 1977), is itself an attempt to raise public consciousness about many of the issues repeated in *The Weather Conspiracy*, I am keenly aware of possible scepticism some might express about one author's seemingly pejorative treatment of a subsequent competitive book. The best that I can do to dispel any such possible suspicion is to state clearly why I believe a "pot boiler" like *The Weather Conspiracy* could really retard the efforts of those who seek to persuade society to anticipate and then hedge against the possibility of future climate-induced catastrophe—a goal that seems common to me and the Impact Team.

Commendably, *The Weather Conspiracy* does bore deeply into many of the issues of future climatic warmings and coolings, but instead of pointing out that either scenario for climatic change could be troublesome since much of human activity, particularly agricultural, is tuned to the present climate, it insists on maintaining the shock effect of the dramatic (the subtitle reads, "The Coming of the New Ice Age") rather than the reality of the discipline; we just don't know enough to chose definitely at this stage whether we are in for warming or cooling—or when. Nor, is *The Weather Conspiracy* alone in choosing sides in a scientific debate which is just not resolvable with present knowledge. Two other recent popular books by non-meteorologists give away their opposing advocacies in their titles: *Hot House Earth* versus *The Cooling*.

The damage to the authors' common cause of action on public policy from all three books is that they have been discredited publicly by many in the scientific community as sensationalist and technically inaccurate. Thus, in the confusing banter among experts—some pushing cooling, some pushing warming—the public and their elected officials usually shrug and say, "Let the scientists study more until they are sure what will happen".

That is precisely one of the greatest inadequacies that governmental institutions exhibit with regard to scientific controversies. They often confuse debates among scientists as a justification for a 'wait and see' attitude on policy considerations. Unfortunately, many controversial, unresolved scientific components of public policy issues, for example, the danger of nuclear power plants, the banning of saccharin or the landing rights of Concorde in New York, are not resolvable before decisions have to be made.

What policy makers need, therefore, is a realistic assessment of what is and isn't known about the science of problems like climatic change, along with some estimates of the vulnerability of different segments of society to a variety of plausible climatic scenarios; and also, some estimate of how long it might take the scientific community to reduce the large uncertainty that exists over the alternative projections of the future.

One hopes we do not need overstated scientific certainty to scare the system into action, for no doubt as soon as one group overstates the strength of scientific evidence to advocate a policy change, someone else advocating an opposing policy will be quick to point out the omissions or errors in the technical evidence, and will challenge the credibility of the original advocate's views—especially their policy options. The result is usually a delay in action, not a speed-up, for the added confusion slows up the process.

If accurate information is a key for society to survive the increasing complexity of the technological props that support its existence, then we must also learn to deal with the bewildering uncertainties surrounding the safety and acceptability of these props, and be willing to make value judgements as to whether we should hedge against the most plausible catastrophes that present knowledge can estimate. If this kind of common sense planning for insurance against plausible nasty surprises in the future can only follow from overstated cases shrieked out of instant books or from television news programmes; if we are unwilling to put in the time to follow in some detail just what science does and does not know about the range of potential technological crises, we will fall into crisis after crisis, under-reacting in advance and over-reacting afterwards.

As I have chosen *The Weather Conspiracy* as a point of departure to argue how books should not treat scientific controversies, let me return to it. Instead of meeting its page one stated purpose: "to inform the public of the true facts about a topic often clouded by fiction, superstition, and alarmist misrepresentation", *The Weather Conspiracy* leads the pack in clouding up further precisely what it is intended to clear. □



# Japan's national R & D programme

John H. Douglas examines the progress of government-sponsored large-scale projects in Japan



A constructive way of using waste: garbage crushed into blocks for use in the building industry.

IF the continued economic success of "Japan Inc." still puzzles the average Westerner, the role played by research and development in fostering the country's industrial achievements remains even more of a mystery. Yet the conduct of research, like that of business, often reflects a typically Japanese character, and examination of research priorities reveals much about Japan's plans for the future.

The outstanding characteristic of the government-business relationship in Japan's "guided capitalism" is a continual search for consensus. When all parties can agree on a course of action, thorough commitment to massive projects can be secured and planning for a very long time-scale is facilitated. Similarly, the strength of Japanese research and development lies in the ability of government, universities and industry to commit themselves to joint effort toward long-term goals.

Not surprisingly, such research by mutual consent is usually limited to pursuing narrowly defined, immediately applicable ends. Inherently the system lends itself to adapting, rather than originating, new technologies, although this pattern may be changing. There are also structural limitations: nearly three-quarters of Japan's total R&D expenditures occur in the private sector (compared to less than half in Britain), which naturally leads to emphasis on applied, rather than basic, research.

Instead of directly sponsoring the majority of research, as in the West, Japan's government has more often played the role of midwife for consensus. But in the mid-1960s it became apparent that some major projects would require more direct government support. The Ministry of International Trade and Industry (MITI) was given the responsibility for sponsoring these large-scale projects, and in 1966 a National Research and Development Programme was established under the ministry's Agency of Industrial Science and Technology (AIST).

The partnership of government, industry and university was thus strengthened to be able to handle even more massive and long-range projects. To qualify for support under the programme, a proposed project had to have "urgent importance for upgrading national industrial standards, promoting efficient utilisation of natural resources, preventing industrial pollution, and so on".

Now, after a decade, some 14 projects have been launched under the programme. Two have been successfully completed: the development of an internationally competitive computer system and of a commercially viable desulphurisation process. One project, the development of a remote-controlled undersea oil drilling rig, was suspended in 1975 for reasons that MITI officials will still not discuss.

## Current projects

A summary of the 11 remaining projects probably offers as clear a guide as any to the major areas of research and development that the Japanese believe should have the highest priority to serve as a base for future industrial and social development. The three outstanding exceptions to this statement include Japan's nuclear energy programme, the search for alternative energy sources called "Project Sunshine" and the country's emerging space programme, which are funded separately.

- **Jet engines:** In terms of anticipated total funding over the expected life of a project (see table below), the largest undertaking in the National Research and Development Programme is an effort to promote a technologically independent jet-engine industry. Specifically, the aim of this project is to produce prototypes of a turbofan jet engine particularly suited to Japan's domestic airlines, whose routes are

relatively short and require frequent stops. An engine with thrust in the 10 to 15 ton range is envisaged, with parts highly resistant to heat fatigue for frequent take-offs and landings.

Tests of a 6.5 ton thrust prototype are now being conducted in a British wind tunnel (there are none in Japan large enough for the task), and if these and later tests prove successful, a model of this engine may be mounted on an aeroplane within three years.

A related project, which may be funded separately and which has not yet been officially announced, is designed to help Japan's domestic airlines: a short take-off and landing (STOL) craft to be fashioned from the American C-1 military cargo plane. STOL capabilities will reportedly be incorporated by mounting jet engines on top of the plane's wings, so the plane could then carry about 125 passengers. The new project is expected to cost a total of about 17,000 million yen.

- **Pattern information processing:** Optical recognition of letters, numbers, *kana* (Japanese syllable characters) and *kanji* (Chinese ideographs) has now reportedly been achieved. MITI officials say the first practical application of the system can be expected within two years, probably in the patent office as an aid to processing applications.

This project also includes several parallel efforts including experiments with artificial intelligence and development of new electronic components. Active research is being sponsored in the fields of magnetic bubble devices, semiconductor lasers, holographic memories, very large scale circuit integration (megabit memories on a single chip) and microprocessor architecture for a variety of applications.

- **Magnetohydrodynamics:** MHD power generation, now the oldest continuing project in the programme, may turn out to be too expensive for even this sort of national effort. One senior MITI official says that MHD offers a "good opportunity for a joint project" with other nations, and he hints that active negotiations toward this end are already in progress.

Present Japanese targets call for testing a 100 kilowatt generator with a copper-iron magnet for 200 hours in 1980. Then, if these tests are successful, a 100 kilowatt generator with a superconducting magnet is planned for 1982.

● **Nuclear steelmaking:** In anticipation of the successful development of a multi-purpose high-temperature gas reactor (HTGR), funded separately, MITI is coordinating the efforts of more than a dozen major companies to produce the components that will be required by a steelmaking system based on the reactor. Some of the major goals of the first phase of this project include development of a 1.5 megawatt heat exchanger loop, alloys and insulation materials capable of handling the 1000 °C helium gas coming from the HTGR.

Parallel efforts include construction of a steam reforming test plant to make a reducing gas from light hydrocarbons, development of an apparatus for charging a shaft-type furnace under the new conditions, and completion of a conceptual design for the proposed system. If phase one is completed on schedule, by 1979, a nuclear steelmaking pilot plant is proposed, to begin operation as early as 1986.

● **Complex manufacturing:** Begun during the current fiscal year, this latest project aims at production of a single machine complex that can handle many successive manufacturing steps—such as grinding, milling, welding, forging and casting—at one location. Computer control and the latest laser technology will be incorporated.

● **New process for producing olefins:** Unsaturated hydrocarbons of the olefin series (general formula  $C_nH_{2n}$ ) form the raw materials for many key petrochemical industries, and the Japanese fear that these industries will be threatened by anticipated shortages of imported naphtha, from which the country's olefin supply is presently produced. Since countries with domestic sources of natural gas or light oils are not so concerned with the naphtha problem, Japan has emerged as perhaps the world leader in developing a technology for producing olefins directly from crude oil, which is more likely to remain available.

During the first stage of the project, which ended in 1973, a test plant capable of treating five tons of crude oil per day was built. During the second stage, a 120 ton per day pilot plant is being constructed and experimental work is proceeding to see if olefins can also be produced from the residual oils left after vacuum refining of crude oil.

● **Resource and energy recovery.** Japan's extremely concentrated population (only about 20% of the country's land is flat) suffers acutely from problems that for many other countries are still only nuisances. The National Research and Development Programme has so far attacked four of these prob-

Japan's National Research and Development Programme		
Project	Anticipated Life	Lifetime Cost (10 <sup>9</sup> yen)*
Jet Engine	1971-1980	25.4
Pattern Recognition	1971-1980	25.0
MHD	1966-1982	18.4
Nuclear Steelmaking	1973-1979	12.3
Complex Manufacturing	1977-1983	12.0
Olefin	1975-1981	10.0
Resource Recovery	1973-1981	9.8
Traffic Control	1973-1978	7.3
Desalination	1969-1977	6.7
Electric Vehicles	1971-1977	5.7
(£1 ≈ 450 yen)		

lems: air pollution, urban waste, traffic congestion and water shortages.

To recover useful materials and energy from urban wastes, two 100 ton per day experimental treatment plants are now being developed. The feasibility of several of the technologies involved has already been demonstrated and the project is expected to be completed successfully within three or four years. Technologies now being considered for practical application include cryogenic shredding of wastes, magnetic and air-stream separation techniques, and internally heated fluidised-bed pyrolysis.

● **Traffic control:** To help drivers cope with some of the world's worst traffic congestion, what is probably the world's largest advanced traffic control experiment is now being conducted in a ward of southwestern Tokyo. In an area of about 30 square kilometers, traffic is being monitored by a highly automated control centre, which advises drivers of road conditions and routing options, through a variety of media.

Some 300 test vehicles have been equipped with a display panel that automatically tells a driver the fastest route to a predetermined destination. Another 1,000 vehicles have been provided with a simpler driving information unit that flashes such messages as "Road Construction Ahead". And three roadside display boards at key locations provide all drivers with notice of road conditions and recommended detours.

● **Water desalination:** For a country so notoriously damp and rainy (with more than twice the annual rainfall of Britain), Japan would seem to have little reason to give such high priority to a project for taking fresh water from the sea. But again because of the extreme concentration of people, water shortages are expected to begin in Tokyo and other major cities perhaps as early as 1980.

The MITI-sponsored desalination project, which ends this year, involved construction of a 100,000 m<sup>3</sup>/day test

plant using multi-stage flash evaporation (MSF) to produce fresh water. As a result of successful experiments at the plant, the Japanese now claim to lead the world in MSF technology, and private companies are exporting commercial desalination plants with capacities of 30,000 m<sup>3</sup>/day.

● **Electric vehicles:** In the other major project to be completed this year, five second-generation electric vehicles (two cars, two trucks and a bus) have been successfully designed, built and tested. MITI officials say the next step toward full commercialisation must be removal of legislative and economic disincentives and further improvement of accumulators for the vehicles.

During the fiscal year 1977, MITI's total expenditure for the National Research and Development Programme, including management cost, was 14,484 million yen (about £32 million). Thus, although the programme was designed to infuse government funds into areas of research that private companies might hesitate to enter alone, public expenditure remain relatively small. Commercial success in any one of the projects could conceivably bring to Japan enough foreign exchange to support the whole programme.

Rather than producing the "spin-offs" that result from open-ended research common in projects sponsored by Western governments, the MITI projects pull along a family of "spin-in" technologies. When, for example, a holographic memory or a new high-temperature alloy is finally developed, an eager market will be waiting. Theoretically, the disadvantage of even such long-range, loosely defined "applied" research is that it will not produce the sort of original breakthroughs that come haphazardly from basic research. So far, however, Japan has had little trouble importing these fundamental advances from abroad. As long as this flow of ideas remains adequate Japan's National Research and Development Programme will remain one of the country's biggest bargains. □



## Sweden debates gene-splicing

For the first time, recombinant DNA research is being publicly debated in Sweden. Some voices are calling for broad understanding and judgement of the ethical, economic and political issues involved, while others are more concerned with the security aspects of a proposed P3 laboratory at Uppsala. At the same time, the Ministry of Education is setting up a one-man committee to see if existing Swedish laws are sufficient for the regulation of such research, or whether new legislation may be needed.

It sounds as though the country has suddenly woken up to find recombinant DNA research sitting on its doorstep. Not so. The previous government was approached about the issues as long ago as 1973, but did not respond. In 1975 Uppsala biologists applied for a P3 laboratory. In the spring of 1976 an eleven-man "Committee Concerning Research with Recombinant DNA" was set up under the auspices of the Natural Science Research Council, the Medical Research Council and the Swedish Cancer Society to ensure that such research was carried out in safety for both laboratory personnel and the public at large. The committee has decided that Sweden's risk classification system should be a combination of the British Williams' guidelines and the United States' NIH guidelines, adopting the stricter elements of each. Although the committee's mandate only covers state-supported research on recombinant DNA—and it must

approve all proposals for such research before they can begin—private industry has voluntarily agreed to follow the same procedure with its proposals.

According to the committee's chairman, Professor Peter Reichard of the Karolinska Institute, recombination of DNA within the same species (which requires only P1 facilities) is being done at the Karolinska Institute and the University of Uppsala, but so far no-one is doing any research needing P3 facilities. That part of the Uppsala group's work which has needed such a laboratory has been done so far at the Pasteur Institute in Paris. Private industry has not yet submitted any proposals to the committee.

Professor Lennart Philipson, who leads the Uppsala group, says he is tired of the controversy aroused by his proposed P3 laboratory. He complains that society's confidence in scientists is waning, and hopes that it will increase with public awareness of what the research involves. His new research programme, in which he will try to develop *Bacillus Subtilis* as an alternative host for recombinant DNA, will not need P3 facilities before 1979. The plans for converting part of an existing building into the new laboratory should be finished before Christmas. Critics of the scheme are demanding that they should be approved not only by the DNA committee, as was the original intention, but also by the local health authorities.

Wendy Barnaby

above the sea surface, shot another 30 m into the air (where some of it was dispersed and evaporated) and then rained down onto the sea. The pipe was capped after seven-and-a-half days. It was assumed that 40% of the oil had evaporated by then, leaving some 9,000 to 13,000 tons on the water. As its viscosity was low, it spread quickly; only 800 to 1,000 tons were recoverable by mechanical means. Chemical dispersants were assumed to increase the damage the oil would do to marine life, so they were applied very sparingly.

A week after the spill, the oil became granulated and later formed tar balls which, in June and July, were drifting over an area of 55,000 km<sup>2</sup> in concentrations averaging 2.5 mg m<sup>-2</sup> sea surface: a level considered to be heavy pollution. Close to the Bravo platform, oil-in-water emulsion under relatively freshly spilled oil was found to be in concentrations of up to about 300 mg/litre water: but further away, it was below levels which have caused acute sublethal effects on the more sensitive stages of fish development in laboratory tests.

At the time of the spill, biological development was in its early spring stage. In a few square nautical miles around and east of the platform, the degree of photosynthesis of the phytoplankton was significantly reduced; but otherwise its development seemed normal. There were few fish, fish eggs and yolk sacs in the area at the time of the spill and for a short time afterwards, and the number and distribution of fish seemed to be unaffected by the oil.

The institute sees several factors accounting for the lack of acute effects. The escaping oil was hot, and this, combined with the action of the wind and the fact that the oil spread quickly over the surface, meant that most of the volatile and toxic compounds were evaporated. What hydrocarbons remained in the sea were diluted by unstable water conditions. Excepting the area close to the platform, the resulting hydrocarbon concentrations in the water column were low.

When the "Tsesis" ran aground in the Stockholm archipelago on 26 October, between 1,500 and 2,000 tons of the 19,000 tons of medium-grade fuel oil she was carrying spilled out into the water. The coast guard managed to contain some of the oil with booms, but winds and currents carried a large part of it to an island and part of the mainland nearby. As the wind blew steadily in the same direction for a couple of weeks after the accident, the oil stayed banked up against the shores, emulsifying down to a depth of 2 to 5 metres.

According to preliminary results from tests carried out jointly by the University of Stockholm's Askoe



*Meteosat, the European Space Agency's meteorological satellite, eventually reached geostationary orbit above the Gulf of Guinea on 7 December, after a series of false starts on the Cape Canaveral launchpad. The picture above is the first taken by the satellite at visible wavelengths, showing cloud cover above Africa and the Atlantic on 9 December 1977.*

## Geography affects oil spill damage

THERE have been two significant oil spills in Scandinavian waters this year: April's Ekofisk blow-out, and leakage from the Russian tanker "Tsesis" which grounded on an unmarked rock in the Stockholm archipelago in October. Although the Bravo blow-out dumped at least six times as much oil into the water as the "Tsesis" did, preliminary research suggests that marine life in the North Sea has not suffered as much as that in the archipelago. According to a Swedish expert, the reason for this is the different geographical factors involved in the two spills.

Preliminary findings published by the Institute of Marine Research at Bergen, Norway, describe the acute effects of the Bravo blow-out on fish and plankton as "small". An estimated 2,000 to 3,000 tons of oil at a temperature of more than 75 °C spurted daily out of an open production pipe 20 m



Laboratory and the Swedish Water and Air Pollution Research Laboratory, the effects so far have been relatively mild in the free water (the pelagic zone) but severe along the coast (the littoral zone). Because the accident happened in autumn, the water was cold and life processes were few and slow. This reduced the effects on both marine and bird life: less than 100 birds so far seem to have been killed. Preliminary estimates show that, within four days of the grounding, the mortality of the zoo-plankton within 400 m of the tanker had significantly increased, and about 35% of the zoo-plankton had either been touched by or ingested oil. What was dramatic, however, was the effect on the littoral zone's bladder wrack community. It was found to be almost completely deserted after the spill. Small crustaceans and molluscs either died or left the area. Laboratory studies have also indicated sublethal effects on mussels and snails.

Dr Olle Linden, who is leading the Swedish investigations, points out that the dilution factor is very important in determining the amount of damage done to marine life by oil spills. Severe ecological disasters are more likely to occur in shallow, enclosed coastal water than in the open sea, where the oil generally disperses quickly. The oil spilled from the 'Tsesis' was pinned by winds against the shore. In the end, it was the geography that made the difference.

Wendy Barnaby

## Correction

The Food and Agriculture Organisation's (FAO) budget for the biennium 1978-79 will be \$211,350,000 not \$237,377,000 as indicated on page 553 of last week's issue.

## UK Agricultural research is blooming

In contrast to the gloomy future foreseen for British science by many of its funding agencies, the UK Agricultural Research Council's (ARC) latest annual report for 1976-77, published last week, is almost cheerful. It reports a small growth for the Council's share of the DES Science Budget for 1976-77 and a period of stability in the Council's dealings with the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Agriculture and Fisheries for Scotland, the Council's main customers under the Rothschild customer/contractor principle.

The ARC's increased share of the Science Vote over the past two years (its grant for 1977-78 was 1.8% up in

## More natural gas for Pakistan

PAKISTAN is the only country in the East that seems likely to cushion the energy crisis by exploiting its natural gas reserves. This has become possible because of two major discoveries of gas reserves within a matter of 12 months. According to Dr Shahzad Sadiq, Chairman of the Oil & Gas Development Corporation (OGDC), Pakistan is now in a position to export gas in liquid form or, as a gas, through overland pipelines. The western part of India is extremely gas-hungry and the possibility of piping gas to those regions is now being actively considered.

When last December a big natural gas reserve was discovered at Dhodak, in north Pakistan, the condensate oil in the reserve stole the limelight. The then Prime Minister, Mr Zulfikar Ali Bhutto, chose to dramatise it by bringing a bottle full of oil to the floor of the National Assembly (The Parliament), and waving it within inches of the face of the Leader of the Opposition. Pakistan, which watched the display on TV, was thrilled. Oil had been struck!

However, the Dhodak find is essentially a natural gas reserve with some 5 million million ft<sup>3</sup> of gas. And with yet another big gas discovery only last month and again in the north (at Pirkoh in Baluchistan) the focus is now on natural gas exploitation. The Pirkoh gas reserves are estimated to match those of Dhodak.

The Chief Martial Law Administrator General Mohammad Zia-ul-haq, who at present heads the government, visited Pirkoh earlier this month to inaugurate the gas-field and formally underline the importance of the new find in the overall economy of the country. Pakistan suffers balance-of-payments difficulties which are

largely due to heavy imports of oil.

At Pirkoh, the OGDC Chairman told General Zia-ul-haq that an investment of \$250 million in the development of newly discovered oil and gas fields could give a return of \$230 million annually. This can be earned in oil import substitution and in export of gas and surplus petroleum products.

The gas fields at Pirkoh and Dhodak are not the largest in Pakistan. The biggest one is isolated at Sui in Baluchistan and has an estimated reserve of 10 million million ft<sup>3</sup>. It has been exploited for the last two decades. Pirkoh and Dhodak are graded next to Sui in reserve contents. The fourth largest gas field, at Mari in upper Sind, has also been exploited for some time; its estimated reserves are some 4 million million ft<sup>3</sup>. Total gas reserves in Pakistan are now estimated to reach 30 million million ft<sup>3</sup>.

The most significant impact of natural gas in Pakistan has been on agriculture. The four big fertiliser factories already in production (largely producing urea, also some ammonium nitrate and ammonium sulphate) entirely depend on the natural gas for the power and feedstock requirements. In addition, four more big fertiliser projects, based on natural gas, are under way. The green revolution that has almost doubled the average yield of wheat, the staple grain, and rice through the introduction of dwarf varieties within the last decade calls for plenty of fertilisers as its most vital input.

As a result of the two natural gas discoveries it should be feasible to set up more fertiliser and cement plants, apart from proposed exports to ease the balance of payment position.

Azim Kidwai

ABRC's sympathies to the work of the Priorities Working Party set up in June 1976 to select areas of basic agricultural research which could be most suitably funded from the DES Science Budget. Under the heading 'plants and soils' it chose crop variability, soil/root relationships and crop bioenergetics as priority topics for research. It has yet to select priorities in 'animals' and 'food' subjects. In line with the choice of priority topics, however, the £300,000 bonus is to be spent on setting up a programme of genetic manipulation in plants in several institutes.

The £800,000 which has been set aside for construction will most probably be spent on the improvement and the extension of existing institutes.

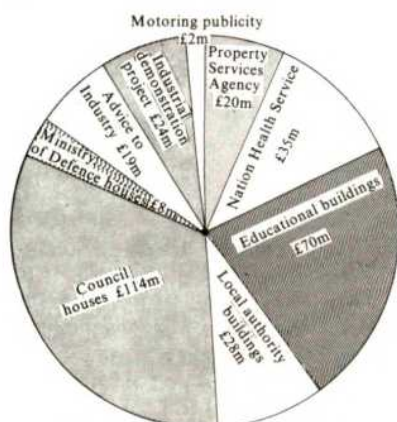
Judy Redfearn

real terms on that for 1976-77), is attributed to a growing appreciation of the need for fundamental research in agricultural and related sciences by the Advisory Board for the Research Councils (ABRC). In particular, the ARC feels that it received sympathetic consideration when the ABRC allocated the extra money set aside for the research councils after the mini budget at the beginning of last month. Of the £4 million to be added to next year's Science Vote, the ARC will get £300,000. And of the £4 million to be spent on new capital work next year it will get £800,000.

Sir William Henderson, ARC Chairman, attributes part of the Council's success in winning the



## Energy conservation comes of age



Expenditure on Energy Conservation  
1978/79 - 1981/82

LAST week's announcement by UK Energy Secretary Mr Tony Benn of a £320 million package of government-sponsored energy conservation measures provided a firm indication that in Britain, as elsewhere in Western Europe, energy conservation has finally come of political age.

At meetings held last month by the Council of Europe in Strasbourg and the European Economic Commission in Brussels, speaker after speaker came to the platform demanding action on conservation with an enthusiasm unknown even six months ago. After the Brussels meeting, Herr Guido Brunner, EEC commissioner for energy, announced that the commission was likely to set up a new directorate concerned with energy conservation. And the German government has since announced a conservation package, including doubling the tax on home heating oil.

The largest part of the British package, which is to be spread over a period of four years, is the £28.5 million a year which is to be spent on providing loft insulation and draught-proofing measures for two million council houses. There will also be considerable sums of money allocated to government departments responsible for buildings.

As far as industry is concerned, a major expansion of information and advisory services will take place at a cost of £19 million over four years. Discussions are taking place with the motor industry about steps to reduce petrol consumption in new cars. And more than £20 million is being provided for an extended programme of demonstration projects, providing examples of the type of savings that can be made by individual companies.

Perhaps the most significant aspect of the British package is the financial investment that it implies in energy conservation measures. Last year a

Government White Paper stated that, while encouraging public sector bodies to finance such measures from their existing budgets, there was "no case for special financial assistance".

In last week's announcement, however, although Mr Benn repeated that programmes would be financed "as far as possible" from savings made elsewhere in existing programmes, over half of the initial £320 million budget is to be "new money". Only £93 million is being obtained from redirecting existing funds, the rest is to come from recycling the savings made from initial stages of the programme.

Another important indicator of government determination is the decision to establish a new Energy Conservation Division in the Department of Energy under Mr Bernard Ingham, for the past four years the department's director of information. The division will be responsible for developing and coordinating UK energy conservation policy.

Energy conservation has thus been clearly placed near the top of the department's agenda, receiving considerably more attention than two years ago when, in spite of the publicity surrounding the "Save It campaign", the House of Commons Select Committee on Science and Technology dismissed the department's conservation efforts as "feeble".

The Select Committee did little to influence the department's strategy, which until now has relied primarily on example and propaganda. Since then, however, at least three factors seem to have contributed to the development of a more aggressive approach towards conservation.

The first of these has been President Carter's continuing determination to pass a wide-ranging energy bill through conference, providing the framework for a coordinated energy policy. A second factor has been the recent upturn in the British economy which, with the economic benefits of North Sea Oil beginning to be felt, have placed the government in a position to allocate financial resources to new programmes.

Perhaps most significant in political terms, however, was the summit meeting of head of state held in London in May. Energy conservation occupied a prominent position in the final communiqué, which stressed the need for "strict conservation measures" to enable an energy market to function harmoniously.

Shortly after the summit meeting, Mr Benn set in motion a major exercise to examine the scope for energy saving across all sectors of the economy. In particular, he asked for quantified calculations of the savings which could be achieved for specific

levels of public expenditure to obtain a substantial reduction in the growth of energy consumption.

Two other aspects added to the packet's political acceptability. The extra work involved in carrying out the massive programme of insulation—a labour-intensive activity—can be claimed to contribute to the general problem of unemployment (even though local governments may balk at some of the administrative implications). And the short-term, quantifiable benefits of conservation measures provide more tangible—and, to governments of short-time, more useful—political capital than measures whose impact can only be seen over ten years or more.

There remain reservations. Some have suggested that the proposed investment in insulation is too small to obtain maximum effectiveness, and that the measures will prejudice a second bite at the cherry. Others have complained that the package applies only to public sector housing, with no comparable efforts in the private sector.

And, of course, there remains the problem of energy conservation in industry, the biggest consumer of energy accounting for 40% of total consumption. Here the government's strategy remains essentially propagandist; two days after Mr Benn's package, Mr Leslie Huckfield, Industry Under-Secretary, claimed that appropriate measures could save industry up to £370 million a year, and that although this would require a capital investment of £560 million, "with such an incentive for reducing costs, they should be an attractive investment".

Industry itself remains sceptical, both about the Government's calculations with regard to what is practically (as opposed to theoretically) possible, and towards the claims of energy conservation as against other demands for capital investment, such as new equipment. An official at the Confederation of British Industry said last week that rather than offering financial incentives to industry for conservation measures, the government should reduce the general level of taxation, and let industrial management decide how to invest the extra money.

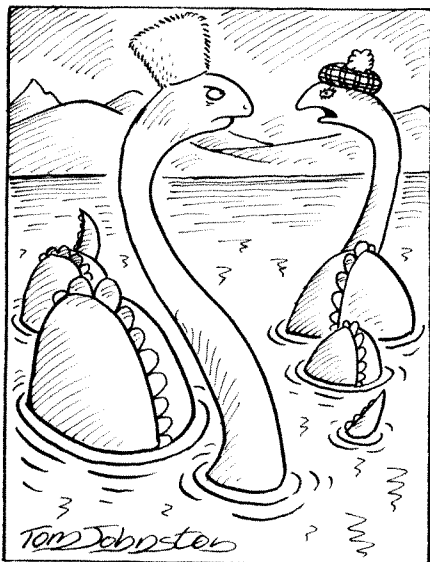
It remains to be seen whether the government will accept this *laissez faire* attitude. The Energy Commission's working paper emphasised that desired changes could not be left to price mechanisms alone, but that mandatory measures were needed to back up financial incentives. Possible measures could include new regulations about building design, petrol consumption of private motor cars, and so on. Mr Benn has demonstrated the size of the carrot; but we have yet to see the size of his stick.

David Dickson



# Soviet New Year Diary

(to be taken with two vodkas and a pinch of salt)



"Better extinct than Red!"

## Koskolteras Rhombopterix?

A Loch-Ness-type monster, 15 m long with a head 2 m long by 1 m wide has been reported in Lake Kos Kol, Kazakhstan. An unnamed commentator on Moscow radio observed that since several "extinct" species have recently been discovered to be still surviving, he considered it quite possible that "unknown creatures of the kind reported in both these lakes" might, indeed, exist. What he did not suggest, however, is any kind of joint Soviet-British (or Kazakh-Scottish) study project. This seems a strange omission; joint scientific research is a major feature in all discussions of mutual cooperation, and a possible monster investigation would enliven what too often become dull routine talks on oil drilling equipment and fertiliser plants. Indeed, should the two creatures prove to be of the same species, an interbreeding project might not be beyond the bounds of all possibility!

## Bubble bath

Small boys on both sides of the space race have always been attracted to the profession of cosmonaut/astronaut—not least because of the obvious impossibility of taking a bath under conditions of weightlessness. Alas for childhood dreams—the days of a lick and a promise with a cologne-dampened washcloth are gone: Soviet technology has succeeded in equipping Salyut-6 with an experimental shower. Enclosed in a special shower cabinet, the cosmonaut is sluiced by a stream of water droplets borne by an airflow; the mixture of air and water being separated by a filter and recycled.

## Un-cooking the books

According to geographer Arkadii Sopotsko, the geographical discoveries on the North West coast of the American continent, attributed to Captain James Cook, should rather be credited to Vitus Bering and Aleksei Chirikov 37 years earlier. The logs of the two explorers were long believed lost, largely because Imperial policy decreed that "Asia eastward cannot end", and evidence to the contrary constituted an important state secret. The missing logs have now come to light in the central Navy archives, and it now appears clear that when Bering, aboard the *Svyatoi Petr* (St Peter) reached the North American coast in July 1741 he mapped and named several features later recorded by Cook. These include the St Athenogenes Ridge (now the Hayday Glacier), Cap St Mary (Cape Suckling), and islands of the Aleutian group which he named respectively for Sts Marcian, Stephen and Abraham.

## To each according to his needs . . .

Aleksandr A. Bulgakov, Chairman of the USSR State Committee for Vocational Training recently announced that the text-book situation has greatly improved during the last year. There is now, he said, "a text-book per pupil in almost every [vocational training] establishment".

## Up the Pole . . .

According to Aisultan Kalybaev, described by TASS as "a young Kazakh scientist", the terrestrial poles can move only by "tens of kilometres", and hence "the hypothesis that 500,000 years ago the North Pole was in the centre of the Pacific Ocean" must be false. This remarkable deduction, "based on the laws of mechanics" was apparently arrived at during research to provide a mechanical and mathematical basis for predicting earth-tremors in Kazakhstan.

Meanwhile a team from Leningrad and Yakutsk have established that the Siberian permafrost is retreating at the rate of 1 to 2 m "along the vertical line" (sic) per century. The melting is not due so much to climatic change as to an inflow of geothermal energy. Whatever the reason, it must be gratifying to the planners responsible for the development of the Soviet Far North to know that they have the backing of a natural process.

## Whose was that baby . . .?

The problem of the sailor, returning from a tour of duty, to be greeted by

an infant unbelievably forward or backward for his official age must be as old as Jason. Soviet seamen need no longer jump to the obvious conclusion, however; a recent broadcast on the seamen's service of Soviet radio reports the case of little Oleg Slonin, who at 14 months knew the entire Russian alphabet "except the hard and soft signs", and could recite 28 poems. The broadcast explained that "such a phenomenon is not very frequent, but is not now altogether rare. It is explained by the fact that, together with a physical acceleration, an intellectual acceleration takes place. Children nowadays have an early intellectual development."

## In dock . . .

A new system for dockworkers, based on the study of "hourly, diurnal, and monthly" biorhythms is under test at the port of Odessa. In the service of productivity and safety, a computer is used to record for each man the "days notable for the maximum decrease of work capacity" and his "highest peak of inattention," according to his "biorhythm-activated schedule". The pattern obtained is used for planning work-schedules: a docker's days off will also be his "off-days".

## Na zdorov'e

The Soviet anti-alcoholism campaign was inaugurated in the middle of the last 5-year plan—to the considerable disruption of targets for potable alcohol, which had suddenly to be replaced by beer and soft drinks. Although a considerable amount of the surplus vodka was unloaded onto the USA in return for pepsi-cola, the problem of hard drinking still remains pressing.

In addition to prosecution and/or compulsory committal for treatment, there is a widespread campaign of publicity and psychological pressure, including publication of crime and fatality statistics associated with the imbibing of alcohol, public rebuke of persistent offenders before their work-mates, and loss of privileges and bonuses. The latter sanctions are common Soviet practice in dealing with antisocial or "uncultured" behaviour—nevertheless, in this case there seems something wrong with the underlying psychology. A common factor in social drunkenness is that a family man living in a one-room apartment cannot conveniently invite his friends home, but must meet them in a bar. To relegate such a culprit to the end of the housing list hardly seems helpful.

Vera Rich



# correspondence

## Conserving uranium

SIR,—The exposition by John Davies (1 December, page 376) of the virtues of the CANDU reactor thorium cycle as a possible alternative to fast reactors does indeed cover matters "being discussed in undergraduate lectures twenty years ago". It does so with a splendid disregard for the present status of the technologies involved.

Recently the experimental fast reactor at Dounreay was shut down after 18 years of successful operation, its original exploratory task complete. An important part of that task was to demonstrate the docile behaviour and ease of control of fast reactors. The UK, France and the USSR are now operating prototype reactors in the 250–350 MW(e) output range. The maturity of the technology is apparent from the fact that the latter two countries have committed the construction of larger units of commercial significance.

The position on the CANDU-thorium fuel cycle is well set out in an authoritative manner in the evidence submitted by Atomic Energy of Canada Ltd (AECL) to the Ontario Royal Commission on Electric Power Planning which was published in April of this year. The section on 'Prospects for future CANDU fuel cycles' brings out the elementary fact that "Thorium is a fertile material but contains no fissile isotope". It is therefore proposed to use plutonium recycled from the existing uranium fuels to commence the cycle. Reprocessing of both uranium/plutonium and  $^{232}\text{Th}/^{233}\text{U}$  fuels is therefore required—a more complex situation than arises with the fast reactor.

It is concluded by AECL that "the overall development and demonstration programme can be completed during the 1990s". This makes it quite clear that we are dealing with a technology which is not yet available even on a pilot scale, and which is unlikely to avoid the problems of MUF (material unaccounted for) and possible illicit diversion to weapons. Incidentally, freshly reprocessed  $^{233}\text{U}$  is not automatically protected by gamma radiation, since this arises from  $^{232}\text{U}$  daughter products, and takes about 10 days to build up to embarrassing levels requiring elaborate remote handling.

The AECL submission to the Royal Commission also discusses the use of accelerators under the heading of

'Electro-nuclear breeding'. Here it is concluded that "if the cost of uranium were to rise substantially, electro-nuclear breeding might be economically justified. In any event such systems will not be required until the advanced fuel cycles are fully established so there is ample time for their orderly development". The quotation places the Chalk River work cited by John Davies in its proper perspective.

In Canada, where there is a substantial investment in manufacturing facilities for CANDU reactors (including the heavy water production plants) the progress to the thorium cycle appears naturally as a logical step. At the expense of an initial increase in the rate of usage of uranium a long term benefit can be obtained. In my view it would not be sensible for this country to follow the same course when, with our starting point, (that is with fast reactor technology available) we are in a position to obtain a much larger energy output from the available stocks of uranium. The thorium reserves would of course also be burnable in fast reactors at a much later date if necessary.

D. HICKS  
*Risley Nuclear Power Development  
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## Censuring repressive regimes

SIR,—The letter from Dr Peto and Professor Doll (1 December, page 384) asks several important questions, especially:

- how can busy scientists obtain a dispassionate assessment of alleged oppressive conditions in countries other than their own?
- What can they do to mitigate such oppression?
- In particular, is it useful to boycott scientific conferences in such a country?

These were just the questions which we examined in our report *Scholarly Freedom and Human Rights*, published earlier this year, in association with the British Institute of Human Rights, by Barry Rose. We concluded that the recent development of international human rights law had, for the first time in human history, pro-

vided a standard frame of reference, internationally agreed, against which the conduct of public authorities in different countries could be objectively measured. We recommended that there should be set up an independent clearing-house which would collect and evaluate information about such conduct in order that scientists could receive objective and impartial assessments on which they could rely, and we suggested the International Council of Scientific Unions and the International Commission of Jurists, jointly, as suitable bodies to undertake that task.

The ICJ has indicated its willingness in principle to take on its part of this function; the ICSU has not yet completed its examination of the proposal. Meanwhile, Peto and Doll were wise to consult Amnesty International: their sources of information are excellent, and their reports command world-wide respect.

There remains the question of the usefulness of boycotts. So far as I know, there is no evidence that public protest, or an effective boycott, have ever been counter-productive. There is some evidence that, on some occasions, they have influenced oppressive regimes for the better (the case of Dr Mikhail Shtern is a recent example). But in the absence of a full understanding of how the internal affairs of oppressive regimes are conducted, or of controlled experiments with matching samples, we can never know for sure.

The best procedure—at least in my view—would be for reputable international scientific bodies to agree in advance not to hold conferences and congresses in countries whose regimes have been clearly and impartially shown to pursue a consistent policy of oppression towards scientists, scholars and other non-violent citizens. There cannot be many countries which could survive such isolation for long.

This is a subject which has caused many scientists many personal and collective problems in recent years. We hope that the study of the underlying principles which is set out in our report can make its contribution to clarifying those problems, and indicate the directions in which their solution may be found.

PAUL SIEGHART

*Council for Science and Society,  
London, UK*

## Catastrophe theory

SIR,—It is not my purpose here to discuss in detail the criticisms of catastrophe theory contained in the review article by H. Sussman and R. Zahler (27 October, page 759). I would like to refer the interested reader, however, to a forthcoming article of mine entitled 'Mathématique et théorisation scientifique' to appear in *Scientia*. I would also like to point out a misquotation by the authors. The classification theorem for the "Cusp catastrophe", erroneously quoted as "Thom's theorem", is in this specific case due to H. Whitney (Mapping of the plane into the plane, *Ann. of Math.* 2, 62, pp. 374–410 (1955)).

RENE THOM

*Institut des Hautes Études Scientifiques, France*

SIR,—I would like to make two criticisms concerning Zahler and Sussman's recent review article on the applications of catastrophe theory (27 October, page 759). The first is that an editorial policy which sanctions the publication of polemical and emotive articles has no place in a scientific journal. In my view, the tone of Zahler and Sussman's comments goes beyond the lively discussions which we all welcome. Despite its many successes, there have been incorrect examples and applications of Thom's theory and extravagant claims for it. These should be assessed carefully and any errors rebutted scientifically. Zahler and Sussman's excessive and misleading criticisms do not help in the proper evaluation of the usefulness of Thom's theory and can only encourage a polarisation of opinion.

My second criticism is of the poor standard of argument and exposition in the paper and some comments on the first two pages follow. First Zahler and Sussman state that the record of legitimate uses of Thom's theory in physics and engineering is poor. But the theory has been applied very successfully in engineering<sup>2,3</sup> and optics<sup>4</sup>.

Next, Zahler and Sussman appear to have missed the real weakness in the claim by Kozak and Benham<sup>5</sup> and as a result much of their criticism is irrelevant. Thom's theory applies to processes governed by a potential function and with at most five controls, and which no matter how slowly the controls cross a threshold, pass from one equilibrium state to another through transitional states which are not in equilibrium. This passage between equilibrium states is continuous and often, though by no means always, very fast, particularly in mechanical cases, but never instantaneous. The discontinuities or 'jumps' arise if only equilibrium states are measured. Thom's theory does not apply if, when the controls are varied slowly enough, the process remains in equilibrium

throughout the transition. Consequently it is quite mistaken to suggest that the theory offers the cusp catastrophe as 'an inevitable, universal paradigm' for any system which exhibits sudden changes associated with two control parameters. Indeed because the process of collagen denaturation described by Kozak and Benham is of this kind<sup>6,7</sup>, Thom's theory does not apply and one cannot expect collagen denaturation to conform to the cusp or any other catastrophe. The arguments based on the van't Hoff equation which Zahler and Sussman make in the section aptly headed 'Confusion about continuity' are quite irrelevant since the equation holds only under equilibrium conditions.

They go on to assert that 'most biological situations which catastrophe theory tries to model' [sic] are 'inherently continuous'. This is certainly true in the sense that the transition from one state to another, no matter how sudden, is still continuous at least above the quantum level, even if the process is not in equilibrium during the transition. It is not true if by inherently continuous Zahler and Sussman mean (as I think the context indicates that they do) that the transition states of such processes are equilibrium states. Genetic assimilation and quantum evolution are two important biological processes which involve transition states which are not in equilibrium. Moreover the concept of fitness and the widely used selection landscape of Simpson and Wright<sup>8</sup> imply that evolution commonly proceeds from one maximally fit form to another through intermediate forms which are not maximally fit, that is not in equilibrium with their environment.

In fact, contrary to Zahler and Sussman's assertions on page 762 about my work, the notions of fitness and selection landscape permit Thom's theory to be incorporated naturally into the analysis of the adaptive response of populations subject to natural selection in slowly varying environments. This has been done for quantum evolution<sup>9</sup>, genetic assimilation<sup>10</sup> and allopatric speciation<sup>11</sup>. Two of these papers contain quantitative predictions for the response of the phenotype, although it is true that the imperfection of the fossil record and the variability in populations present great practical difficulties. However, these are problems which are common in applying mathematics to biology and are not peculiar to catastrophe theory.

Similarly in his work, Zeeman assumes a gradient system (a common enough practice in biology) so that Thom's theory can be applied. Moreover he makes many quantitative predictions which depend on the properties of the cusp catastrophe. There are a number made in 'Primary and secondary waves in developmental biology'<sup>12</sup>, cited by Zahler and Sussman, particularly in §9

and §15. It is a limitation of the theory that some of these predictions can only be made to first order, as Zeeman makes clear in the second sentence of §9. Incidentally Zahler and Sussman's remarks about Zeno's paradox suggest that they have not appreciated this nor that at its vertex the cusp is flat. These predictions, in contrast to Zahler and Sussman's assertions, are not contained in the data, not independent of the theory and not just wrong. They need, as Zeeman suggests explicitly, to be tested experimentally and Zahler and Sussman's description of these predictions as 'purely unverified hopes' is a gross misrepresentation of what Zeeman has said, as reference to the paper just cited will show.

Zahler and Sussman do not make it clear that in spite of the underlying continuity, many important biological phenomena, such as somites, boundaries between different tissues, etc., are discrete and discontinuous. This provided a considerable stimulus to Thom's thinking and it is no accident that his theory offers a possible way of handling such phenomena mathematically. The most profitable procedure is to test the predictions which follow from applications of the theory and to see whether its geometrical framework gives useful insights.

A general comment is that the accomplishments or otherwise of the theory can be better assessed if a clear distinction is made between an example and an application, and between an explanation and a description.

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1. Zahler, R. S. & Sussman, H. J. *Nature* 269, 759 (1977).
2. Thompson, J. M. T. *Z.A.M.P.* 26, 581 (1975).
3. Thompson, J. M. T. *Nature* 254, 392 (1975).
4. Berry, M. V. & Nye, J. F. *Nature* 267, 34 (1977).
5. Kozak, J. J. & Benham, C. J. *Proc. Natn. Acad. Sci., U.S.A.* 71, 1977 (1974).
6. von Hippel, P. H. & Kwok-Ying Wong *Biochem.* 1, 664 (1962).
7. von Hippel, P. H. & Kwok-Ying Wong *Biochem.* 2, 1387 (1963).
8. Simpson, G. G. *The Major Features of Evolution* Columbia University Press, 1953.
9. Dodson, M. M. *Evolut. Theory* 1, 107 (1975).
10. Dodson, M. M. *Math. Biosc.* 28, 264 (1976).
11. Dodson, M. M. & Hallam, A. *Amer. Nat.* 111, 415 (1977).
12. Zeeman, E. C. in *Lectures on Mathematics in the Life Sciences* 7, 69 (1974).

## Colour genes

SIR,—Our ignorance of the genetic basis of skin colour is not as profound as implied in Dr Bowne's letter (13 October, page 556). Nor do we have to assume the existence of unknown genes closely linked to colour genes in order to explain other traits that might be associated with skin colour, for there is evidence suggesting that the vast majority of pigmentation loci are probably pleiotropic (Deol, M. S. *Ann. Hum. Genet.*, 38, 501 (1975)).

Dr Bowne's views on intelligence are, of course, unexceptionable.

Yours faithfully,

M. S. DEOL

*University College, London*

# news and views

## Cell culture studies provide new information on tumour promoters

from I. Bernard Weinstein and Michael Wigler

MANY factors influence tumour incidence. Among these are the chemical carcinogens which, as a class, may act as mutagens. Tumour promoters are factors of an entirely different nature. They can be defined as agents which increase tumour incidence when administered after a suboptimal dose of carcinogen, but which are not in themselves carcinogenic. Recently, reports on the effects in cell culture of the most potent known promoting agents, the phorbol diesters, have raised expectations that an understanding of the action of these compounds *in vivo* is near.

The existence of tumour promoters was most clearly demonstrated more than 30 years ago by Berenblum and others who found that an oil extracted from the seed of *Croton tiglium* L. dramatically enhanced the incidence of skin tumours in mice pretreated with carcinogens. Croton oil was not by itself tumorigenic. The exposure of skin to carcinogens, was called 'initiation' and the subsequent step, the repeated application of croton oil or other agent, which elicited the growth of tumours from initiated cells, was called 'promotion'. Early work in this field established that the initiated state is extremely stable, extending perhaps for the lifetime of the animal. In fact, mice can be initiated with carcinogens *in utero* and, at some time after birth, tumours elicited by topical application of promoters (Goerttler & Loehrke *Virchows Arch.* **A372**, 29; 1976). Promotion generally leads to the development of benign papillomas; prolonged exposure results in the appearance of carcinomas. In 1968, the active principles of croton oil were isolated and their structures elucidated (Hecker *Cancer Res.* **28**, 2338; 1968; Van Duuren *Prog. exp. Tumour Res.* **11**, 31; 1969). They are fatty acid diesters of a tetracyclic plant diterpene alcohol, phorbol. Macrocyclic plant diterpene

esters of related structure, some of which are active as tumour promoters, are widespread in the plant kingdom. Other compounds and crude extracts, notably phenols, anthralin and extracts of tobacco tar, have tumour-promoting activity when tested on mouse skin, but no agents studied so far approach the phorbol esters in potency.

Recent observations on the action of the phorbol esters *in vitro* can be subsumed under two principles. The first is that nanomolar concentrations of tumour-promoting phorbol esters (but not their inactive analogues) induce changes in cultured cells which resemble those seen on transformation with either chemical carcinogens or tumour viruses, and further enhance the expression of these transformation-specific phenotypic features in already transformed cells. In chicken embryo fibroblasts (CEF), phorbol esters alter cellular morphology (Driedger & Blumbers *Cancer Res.* **37**, 3257; 1977), increase plasminogen activator synthesis (Wigler & Weinstein *Nature* **259**, 232; 1976), alter the composition of glycopeptides obtained from cell membranes (Weinstein *et al.*, in *Origins of Human Cancer*, Cold Spring Harbor Symp. quant. Biol., in the press), increase deoxyglucose uptake (Driedger & Blumberg *op. cit.*), and cause loss of LETS, the large external transformation sensitive protein (Blumberg *et al.* *Nature* **264**, 446; 1976). Similar changes are also seen when CEF are transformed by Rous sarcoma virus (RSV). The transformation mimetic effects of the phorbol esters are not all secondary to growth stimulation since they occur in CEF under conditions where growth stimulation does not occur. Phorbol esters enhance the expression of transformation-associated properties in transformed cells. The clearest example of this is the combined effect of the Rous sarcoma genome and phorbol esters on plasminogen activator production in CEF. Each factor alone increases the synthesis of the enzyme. Together they have a multiplicative effect (Weinstein *et al. op. cit.*). Synergistic action is also observed in alterations of the mem-

brane glycopeptides of CEF (Weinstein *et al. op. cit.*). O'Brien and Diamond have reported similar findings with respect to phorbol ester-induced ornithine decarboxylase levels in chemically transformed and normal hamster cells (*Symposium on Mechanism of Tumour Promotion and Cocarcinogenesis* Gatlinburg, 1977, in the press). If phorbol esters can enhance the expression of transformed properties *in vitro*, perhaps they can do so *in vivo* and thus provide a preferential growth advantage to latent tumour cells.

The second principle is that the phorbol esters reversibly inhibit terminal differentiation. This effect was noted independently in three laboratories using two systems: chicken embryo myoblasts undergoing myogenesis (Cohen *et al.* *Nature* **266**, 538; 1977), and Friend erythroleukaemia cells (FEC) undergoing either spontaneous or induced erythroid differentiation (Rovera *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 2894; 1977; Yamasaki *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 3451; 1977). These initial observations have been extended to the differentiation of 3T3 cells to lipocytes (Diamond *et al.* *Nature* **269**, 247; 1977), chondrogenesis of chicken embryo chondroblasts (Pacifici & Holtzer *Am. J. Anat.* **150**, 207; 1977), and differentiation of neuroblastoma cells in culture (Ishii *et al.* *Science*, in the press). These observations and their generalisation, if applicable to mouse skin, provide a seductively simple interpretation of initiation and promotion. The stem cells for epidermis are continually dividing, yet the tissue as a whole is in a state of balanced growth, and a stable stem cell pool size is maintained. This is possibly achieved by a regular asymmetric division of the stem cell: one daughter cell becoming a stem cell and one daughter cell terminally committed to differentiate, irreversibly losing its growth potential. If a potential tumour cell were restrained to the stem cell mode of division, it could not increase its proportion in the stem cell pool. If, however, the stem cell division mode were interrupted by the action of a promoting agent, a potential tumour

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cell could increase its proportion in the cell population and give rise to a tumour. Two cautions must be noted in the exercise of this hypothesis. First, phorbol esters greatly potentiate transformation by chemical carcinogens in cell culture systems (Lasne *et al.* *Nature* **247**, 490; 1974; Mondal *et al.* *Cancer Res.* **36**, 2254; 1976) where the stem cell concept may not be applicable. Second, phorbol esters have not been shown to inhibit terminal differentiation of mouse skin.

The two generalisations of recent

data on the action of phorbol esters in cell culture, that they mimic and enhance the tumour phenotype and that they inhibit terminal differentiation, are preliminary but provocative. The factors which determine tumour incidence, latency and rates of tumour progression are largely unknown. Studies on the cellular and molecular basis for the action of the phorbol esters and similar compounds may clarify these aspects of carcinogenesis and suggest novel approaches to the control of neoplasia. □

## Limits to similarity among coexisting competitors

from Henry S. Horn and Robert M. May

ABOUT 50 years ago, the theoretical work of Lotka and Volterra and the laboratory experiments of Gause, Park and others led to the formal enunciation of the 'competitive exclusion' principle: species that make their livings in identical ways cannot coexist. Although this principle may appear fundamental, it is not really very helpful. As stressed by Hutchinson, in his classic 'Homage to Santa Rosalia, or why are there so many kinds of animals?' (*Amer. Nat.* **93**, 145; 1959), the meaningful question is rather how similar can species be, yet persist together? What are the limits to similarity?

Most competitive situations involve many different ecological factors, which are too difficult to disentangle with present methods. But insight can be gained from those special situations where competitors sort themselves out

mainly along a single resource axis, such as food size or foraging place. Hutchinson catalogued many examples, drawn from both vertebrates and invertebrates, of sequences of competing species in which the average individuals in successive species have weight ratios around 2. This implies ratios of about the cube root of 2, or 1.3, between typical linear dimensions (for example, beak length) of successive species. Many other examples have subsequently been tabulated, particularly for birds, lizards and frogs, by MacArthur, Diamond, Cody, Schoener, Pianka, Toft and others. In an extensive study of guilds of birds on various West Indian islands, Faaborg (*Amer. Nat.* **111**, 903; 1977 and in the press) has shown that the weight ratio of roughly 2 holds for passerines, but that non-passerine sequences typically have smaller weight ratios.

Recent studies of the workings of the rule among congeneric sequences of invertebrates tend to be more equivocal. Uetz (*J. Anim. Ecol.* **46**, 531; 1977; see also Enders, *Environ. Entomol.* **5**, 1; 1976) has shown that part of the structure of his guild of 10 species of wandering spiders in an oak-maple woodland in the eastern United States conforms to the 1.3 ratio; some of the species with very similar sizes further subdivide their habitat by flourishing at different times in the season. Similar stories are told of carabid beetles and guilds of spittlebugs (by Southwood and Halkka, respectively, *Royal Entomological Society Symp. on Insect Faunal Diversity*, Imperial College, September 22–23, 1977). Again the 1.3 length ratio plays a part for those species that are very similar in other respects.

The magic 1.3 ratio is no newcomer to the biological literature. Dyar (*Psyche* **5**, 420; 1890) long ago noted that successive larval instars of many insects have weight ratios of 2, and linear ratios of 1.3. This provoked much discussion, which seems to have been forgotten, and even speculations that the underlying mechanism was a doubling of the number of cells between instars (later shown to be not generally the case; for a review, see Bodenheimer, *Q. Rev. Biol.* **8**, 92; 1933).

This empirical relationship is still not understood. When the size of food is considered, various lines of argument lead to the expectation that the average difference in food size between two species should not be appreciably less than the characteristic range of food sizes utilised by either species. This, however, does not explain why the typical intraspecific range of food items spans a weight ratio of around 2.

Before too much time is spent theorising about the Dyar-Hutchinson rule, it should be noted that it appears in many other contexts. In the conventional ensemble of recorders, the lengths of soprano, descant, treble, tenor, bass and great bass are in the ratios 1.2, 1.5, 1.3, 1.6, 1.4. For the consort of crumhorns, the heights of descant, treble, tenor and bass go as 1.2, 1.5, 1.4. The usual consort of viols have typical lengths of treble (61 cm), tenor (86 cm) and bass (110 cm) in the ratios 1.4, 1.3; the alto (70 cm) and viola d'amore (85 cm) are competitively excluded and are relegated to solo roles.

The four string instruments of the modern orchestra—violin, viola, cello, double bass—are grossly discrepant with the rule, having length ratios 1.2,

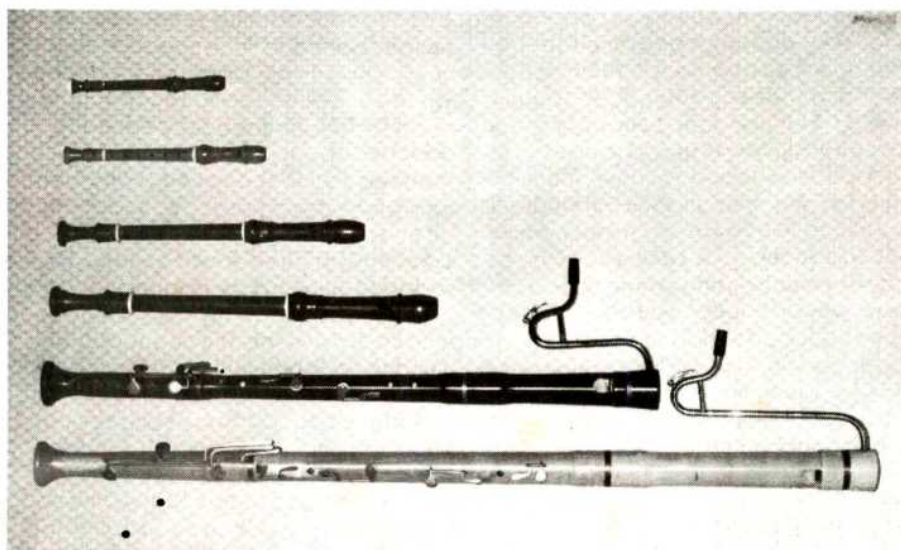


Fig. 1 The conventional ensemble of recorders, whose lengths roughly obey the '1:3 ratio rule'.

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1.8, 1.7. This apparent counter-example is instructive. Taking advantage of acoustic experiments begun 40 years ago (by Saunders, a Harvard physicist), Carleen Hutchins has designed and built a 'new violin family'. This is a unique collection of eight stringed instruments, each of which has the general shape common to members of the conventional violin family. The new violin family has resided in London since it first arrived there in 1974, and it was recently the central attraction at a meeting of the Catgut Acoustical Society (see Darius, *New Scientist* **175**, 21 July; 1977). Darius writes 'Not since the consort of viols has there been an ensemble of acoustically matched stringed instruments . . . What distinguishes the new instruments is the rich, even tone quality . . . The ensemble of these new instruments particularly shines in intrapuntal writing'. For the family, the lengths of sopranino, descant, treble, alto, tenor, baritone, bass and contrabass are in the approximate ratios 1.2, 1.2, 1.3, 1.3, 1.3, 1.3, 1.3.

Ranging yet more widely, we note that the larval instars of children's tricycles and bicycles have typical wheel diameter ratios of 1.3, 1.2, 1.3, 1.2; the adult stages segregate their niches by differences in gear-shift rather than in wheel diameter. One manufacturer's set of five iron skillets gave size ratios of 1.2, 1.2, 1.2, 1.3.

In short, the Dyar-Hutchinson-Hutchins rule may well derive from generalities about assembling sets of tools, rather than from any biological peculiarities. □

## Antimatter

from P. I. P. Kalmus

THE strong and electromagnetic interactions between two antiparticles should be the same as those between corresponding particles. Thus antinucleons should bind together to form antinuclei, and these could capture positrons to form antiatoms. This suggests the intriguing possibility that bulk antimatter might exist in the Universe. This would be exactly as stable as matter, provided that it was isolated. Searches for cosmic antimatter have given negative results, but have hitherto been limited to two methods. A new method of distinguishing between remote matter and antimatter has been proposed recently by J. G. Cramer and W. J. Braithwaite (*Phys. Rev. Lett.* **39**, 1104, 1977).

We would not be able to distinguish between the spectrum of an antistar

and an equivalent star, since the atomic energy levels would be identical and any photon reaching us is its own antiparticle. One way of testing the antimatter hypothesis would be the detection of annihilation when opposite regions collide. An electron and a positron at rest annihilate predominantly into two photons. These must be emitted in opposite directions in the centre of mass each with 0.511 MeV, the rest mass of the electron. The low energy proton-antiproton annihilation is mainly into more than two pions which are therefore not kinematically constrained to be monoenergetic. The decay of the neutral pion gives rise in its centre of mass to two monoenergetic photons of 67.5 MeV, but these would be greatly Doppler-smeared by the relativistic motion of the pion, and would give a fairly broad spectrum with a peak at around 100 MeV. This has not been observed in gamma ray astronomy, and neither has the distinctive 0.511 MeV line. However, H. Alfvén has claimed that this does not exclude the existence of antimatter. It is possible that annihilation would not occur with high efficiency because the tenuous regions of interstellar gas will come into contact first and the resultant pressure of annihilation radiation might keep the matter-antimatter regions apart. Moreover, this initial contact might well be between relativistic electrons and positrons which would not give the 0.511 MeV line.

Convincing evidence for cosmic antimatter would be its detection in the primary cosmic radiation. The detection of anticarbon or heavier antinuclei would indicate the existence of nucleosynthesis in antistars. Several groups have searched for such antiparticles including G. F. Smoot and colleagues who have flown a superconducting-magnet particle spectrometer on a balloon. No antiprotons or antinuclei have been found, the upper limit to their abundance being less than  $10^{-4}$  of the corresponding nuclei. It seems unlikely therefore that significant amounts of antimatter exist in our Galaxy.

Fusion processes and nucleosynthesis in stars systematically convert protons into neutrons and release neutrinos. The corresponding reactions in antistars would release antineutrons and so in principle suggest a test for antimatter. However, as solar neutrinos are only just detectable by present techniques, neutrino astronomy of specific distant objects does not seem to be imminent.

The method proposed by Cramer and Braithwaite makes use of the violation of parity and of particle-antiparticle conjugation in the above weak interactions. These processes, in stars,

involve the emission of positrons or the capture of electrons. The positrons are preferentially in a right helicity state, that is, their spins are aligned along their direction of motion. If their emission is accompanied by inner bremsstrahlung or if they produce external bremsstrahlung when slowing down in matter, the helicity will be transferred to produce right-circularly polarised photons. Annihilation of the moving positrons by stationary electrons will cause the forward-going of the two photons, which carries most of the energy, to have the same helicity as the positron. Finally, internal bremsstrahlung which accompanies electron capture will also have a preferred right helicity.

The equivalent reactions in antistars would convert antiprotons into antineutrons and produce electrons or capture positrons. Following the above arguments, bremsstrahlung and annihilation will result in the emission of left-circularly polarised gamma rays.

The question arises as to whether such polarised radiation is actually observable. Normally the nuclear reactions take place in the core of the stars, and the radiation may be absorbed and re-emitted an enormous number of times before escaping from the surface. It seems most unlikely that significant traces of the original helicity will remain in starlight. However, a supernova, even one located in another galaxy offers the possibility of observing this helicity. This type of explosion is believed to occur when fusion in a massive star has terminated with the production of  $^{56}\text{Ni}$  at the peak of the binding energy curve. The  $^{56}\text{Ni}$  decays into  $^{56}\text{Co}$  and then into  $^{56}\text{Fe}$  by electron capture and positron decay. Assuming that the stellar envelope blows off during the supernova explosion, much of the released photon energy occurs at the surface. Also, the positrons are emitted in heavy medium, namely iron rather than hydrogen, which would enhance the probability of bremsstrahlung by a factor 676, the ratio of the squares of the nuclear charges.

The authors estimate the photon flux from supernova in which 0.14 solar masses of  $^{56}\text{Ni}$  is released at a distance of 1 Mpc. In a period of 1 year starting 2 weeks after the initiation of the supernova is seen, a 1 m diameter polarimeter would receive about  $3 \times 10^5$  photons from inner bremsstrahlung, having mean energy 0.2 MeV and 25% polarisation, and possibly a factor  $10^2$  to  $10^3$  more photons from external bremsstrahlung. The helicity of gamma rays has been determined by laboratory experiments by their differential scattering from polarised electrons, but polarimeters



for use in space, and preferably with higher analysing power, would have to be developed if gamma-ray astronomers hope to find antimatter by this method.

## Manipulation of industrial microorganisms

from Juan-Francisco Martín

The fifth FEMS Symposium entitled Antibiotics and other Secondary Metabolites: Biosynthesis and Production was held on 14–16 September at the Ciba-Geigy Research Laboratories in Basel (Switzerland) under the auspices of the Federation of European Microbiological Societies.

WHY microbial strains used in industrial processes produce such large amounts of a particular metabolite in spite of the strict control of metabolism existing in most microorganisms, and how it is possible for scientists to improve the production of pharmaceuticals such as antibiotics, ergot alkaloids and so on, was the main subject of the symposium. One of the great advantages of this meeting was the participation of scientists from the academic world and industry both as speakers and in the audience. The limitation usually imposed by industrial secrecy was not so evident in this meeting and the flow of scientific knowledge in the lectures and discussions as well as in the corridors was quite free.

New approaches to the search for new metabolites of industrial importance were discussed in the first lecture by H. Zähner (Tübingen). Some of the new techniques he suggested such as 'the chemical test systems' where the search for totally new antibiotics is based on screening unusual metabolic products following feeding of the culture with fluor or sulphur radioactive label, were compared with more classical approaches in the search for new drugs such as the tests of antimicrobial and other biological activities or the use of cell free test systems for highly specific activities. It was the feeling of most participants that while the search for unique chemical structures may be useful, more rapid progress can be made using specific 'in vitro' assays to look for inhibitors of those enzymes

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## Coupled transcription/translation

from Pamela Hamlyn

OOCYTES isolated from the ovary of the frog *Xenopus laevis* made their reputation as a valuable tool for molecular biology when they were used to settle disputes concerning the function of the poly(A) tail—the string of adenylic residues added post-transcriptionally to eukaryotic mRNA. Translation of mRNAs into protein in heterologous cell-free systems proceeds for a few hours at most, compared with several days when mRNA is injected into oocytes and so it is not surprising that only in the oocyte translation system was it shown conclusively that the poly(A) tail ensured the stability of mRNA (Huez *et al.* *Eur. J. Biochem.* **59**, 589; 1975).

Although the translation of mRNA into protein has proved to be reasonably amenable to study in cell-free systems the faithful transcription of RNA from DNA has been difficult to reproduce *in vitro*. It now seems that the use of oocytes will have an important role in elucidating the mechanism of transcription and other related problems of gene action, particularly its control.

Recently Gurdon, De Robertis and Partington (*Nature* **260**, 116; 1976) demonstrated that nuclei from human tissue culture cells continue to function (synthesise RNA) when injected into oocytes. Further work from the group showed that intact nuclei were not necessary, but that isolated DNA injected into the nucleus of oocytes could be transcribed into RNA (Mertz & Gurdon *Proc. natn. Acad. Sci. U.S.A.* **74**, 1502; 1977). Several DNAs were found to be effective, but most of the experiments were done with simian virus 40 DNA. It is important to be certain that the new RNA synthesised is transcribed from the SV40 DNA and is not new oocyte RNA transcribed in response to the foreign DNA. To achieve this the authors compared the RNA with that

transcribed when SV40 is growing in its normal host (monkey cells) and found that they were the same size and hybridised to the same region of the viral genome. The same group has now produced much better evidence, not only that the RNA is viral in origin, but also that it is being transcribed correctly. De Robertis and Mertz report (*Cell* **12**, 175; 1977) that they are able to detect specific viral proteins in the oocytes after injecting SV40 DNA into the nuclei. The extra proteins were detected by displaying the total cell protein using the 2-dimensional polyacrylamide gel electrophoresis separation method of O'Farrell. If an inhibitor of SV40 DNA transcription was injected together with the DNA the new proteins did not appear and, most convincing, if SV40 mutants, which code for smaller viral proteins in their normal host, are injected into oocytes the new proteins produced in the oocytes are correspondingly smaller. These results clearly establish that the viral DNA is correctly transcribed in oocytes.

The demonstration that the RNA can be translated into the expected protein is good evidence that the initial transcription was biologically significant—starting and stopping at the right place. It is exactly this kind of precision which has been difficult to achieve in *in vitro* cell-free systems and which is essential for detailed experiments on the control of gene action.

De Robertis and Mertz have also shown that *Drosophila* histone genes cloned in a plasmid have produced 'histone-like' protein in the oocyte coupled transcription-translation system indicating that this technique will be of general application in the study of the control of genetic expression.

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which are known to be involved in the synthesis of essential microbial macromolecules or in animal cell transformation in cancer formation.

The denomination of the so-called 'secondary metabolites' was the subject of an interesting discussion. It was felt that although these compounds present some peculiarities (for example, their chemistry is unusual, they seem to have no essential role in the survival of the producer strains since the ability to produce them is easily lost by mutation, and their production is restricted to certain taxonomic groups), there are

no major biochemical differences in the biosynthesis of these metabolites to justify a separate grouping under the name of 'secondary metabolites'.

The new approaches to the biological and bioengineering aspects of fermentation development were presented by J. F. Martín (Salamanca) and M. Kuenzi (Basel) respectively. The general feeling was that we are on the threshold of great advances in the field of genetic and biochemical manipulation of industrial microorganisms, whereas new developments in bioengineering, such as programmed sub-



strate feeding and the development of new methods of estimation of fermentation parameters will occupy a second place in improving yields in antibiotic production. Martin discussed new advances in gene amplification using molecular cloning techniques, and alteration of the regulatory mechanisms controlling antibiotic production, namely: induction, carbon and nitrogen catabolite regulation, feedback regulation and phosphate (energy charge) regulation. A large number of studies including those presented at the meeting by Z. Vanek (Prague) on the phosphate regulation of tetracycline biosynthesis and H. Pape (Münster) on the carbon catabolite regulation of tylosin biosynthesis, led to the conclusion that deliberate genetic removal of regulatory mechanisms involved in antibiotic biosynthesis is a useful tool to increase antibiotic production in the immediate future. On the other hand, there was a lively polemic on genetic engineering as a tool for the amplification and transfer of genes coding for antibiotic synthesis. While some scientists considered feasible, and perhaps convenient, the possibility of transferring the capability to produce antibiotics to microorganisms other than the natural producer, others contended that it may not even be desirable, let alone feasible, bearing in mind that so little is known about the genes for antibiotic biosynthesis.

Recent progress in the area of biosynthesis of specific groups of antibiotics (tetracyclines, rifamycins and macrolides) were described by Vanek (Prague), G. Lancini (Milano) and H. Grisebach (Freiburg) respectively. Also, advances in the field of  $\beta$ -lactam antibiotics were discussed in detail. E. P. Abraham (Oxford) presented recent results from his laboratory and from others on the complexity of the stereochemistry of ring-closure in the formation of penicillins and cephalosporins. New genetic and regulatory approaches to the development of penicillin production were reported by C. Ball (Ulverston). J. Nuesch and his coworkers H. J. Treichler and M. Liersch (Basel) reported unpublished results of his group at Ciba-Geigy on the methionine biosynthetic pathway in *Cephalosporium acremonium* that provide insight into the precursor and regulatory effect of methionine stimulation of cephalosporin production. All these reports in the field of biosynthesis and regulation of  $\beta$ -lactam antibiotics together with the discovery of new  $\beta$ -lactam antibiotics and  $\beta$ -lactamase inhibitors produced by species of *Streptomyces* and *Nocardia* (the cephamycins, nocardicin, thienamycin, clavulanic acid) point to a renaissance of interest in this interesting group of antibiotics.

Secondary metabolites with no antibiotic activity such as the alkaloids were also considered. Advances on the chemistry, biochemistry and the cell fusion of producer *Claviceps purpurea* strains using protoplasts were reported by Groger (Halle), C. Spalla (Milano) and H. Kobel (Basel). New examples of the use of microorganisms in the specific biotransformations of chemicals and pharmaceuticals were described by K. Kieslich (Berlin) and H. Leuenberger (Basel). These once more illustrate the great possibilities, the advantages and the limitations, of enzymatically catalysed reactions for the modification, as well as the total synthesis of natural products. □

## Nuclear quadrupole resonance spectroscopy

from J. A. S. Smith

The Fourth International Symposium on Nuclear Quadrupole Resonance Spectroscopy was held on 13–16 September, 1977 at the Takarazuka Hotel, near Osaka, Japan. The local Chairman was Professor H. Chihara of Osaka University.

THESE symposia review progress in a branch of radiofrequency spectroscopy very much concerned with structure and molecular motion in solids. There was an air of practicality about the proceedings of this year's conference, appropriate in the light of the ideas of the 8th century Japanese monk, Saicho, who considered that scholars should 'serve in such undertakings which benefit the nation.' The practicality appeared almost immediately in the description of a precise nuclear quadrupole resonance thermometer whose absolute accuracy was claimed to be  $\pm 2$  mK (A. Ohte, Yokagawa Electric Works).

The opening paper was given by E. L. Hahn (University of California, Berkeley), whose laboratory over the past 15 years has done much to stimulate the development of new instrumental techniques in radiofrequency spectroscopy; his paper was concerned with the double resonance detection of  $^2\text{H}$  quadrupole resonance in solids, with the minimum possible degree of enrichment—in this case, 1%. Such a proposal would have been unthinkable some 10 years ago, and

illustrates the extent to which double resonance techniques have developed in recent years. In the following papers, the emphasis was very much on consolidation and application of these advances, from a triple resonance detection method for  $^{17}\text{O}$  in natural abundance (R. Kado, Kyoto Sangyo) to detailed studies of doped alkali-halide crystals (T. Taki, Tokushima). Later papers on the instrumental side laid much stress on pulsed techniques and R. A. Marino (Hunter College, CUNY, New York) described a particularly elegant pulsed system with Fourier transform capabilities for  $^{14}\text{N}$  quadrupole resonance detection, which is now being developed commercially. Together with the development of such instruments, the increasing use of relaxation measurements in chemical and physical problems was very obvious, with H. Chihara reporting on molecular motion in 1,2-dichloroethane, R. Hewitt (University of California, Riverside) on  $^{121}\text{Sb}$ ,  $^{123}\text{Sb}$  relaxation in the metal, A. Colligiani (CNR, Pisa) on  $^{14}\text{N}$  relaxation in benzonitriles, and Y. Abe (Tsukuba) on a careful investigation of structure and motion in hydrazine. Solid state effects were the subject of considerable interest with a survey by G. K. Semin (Institute of Organo-Element Compounds, Moscow) of the wide-ranging studies of electric field effects made by his research group in recent years, and a report by T. Kichi (Osaka) on intermolecular interactions in  $\alpha$ -ICl. Another current field of interest is the potential application to biological problems, a field as B. Lindman (Lund) showed where measurements of quadrupole relaxation times in solution have contributed much new information on ion binding in model membrane systems.

In inorganic applications, much new and important work was also presented. T. C. Waddington (University of Durham) reviewed recent work from his laboratory on the  $\text{ICl}_4^-$  and  $\text{AuCl}_4^-$  ions, which seem to show, in many of their salts, a kind of *trans* influence in their interionic interactions.

Several new Zeeman studies were reported, among which may be mentioned that of H. Negita (Hiroshima) on  $^{81}\text{Br}$  quadrupole resonance in  $\text{NaAl}_2\text{Br}_7$ , in which the bridging  $^{81}\text{Br}$  frequencies were definitely assigned; in an entirely independent set of experiments, A. Weiss (Darmstadt) had also made the same assignment by  $^{81}\text{Br}/^{27}\text{Al}$  double resonance (SEDOR) experiments, distinguishing between bridging and terminal signals by comparing their response to  $^{27}\text{Al}$  irradiation. Fine structure of the  $^{127}\text{I}$  resonance due to In-I spin-spin coupling was reported in  $\text{In}_2\text{I}_6$  (K. Yamada, Hiroshima) and theoretical analysis suggests that the  $J$ -tensor is not axially symmetric. □

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# International histocompatibility testing workshop 1977

from F. Kissmeyer-Nielsen

The seventh (and largest) International Workshop in Histocompatibility was held in Oxford on 4-10 September, 1977. It was organised by Professor W. Bodmer (University of Oxford) with the assistance of Dr P. Morris (Oxford), Professor R. Batchelor (Queen Victoria Hospital, East Grinstead), Dr H. Festenstein (London Hospital Medical College) and Dr J. Bodmer (Oxford). The proceedings will be published by Munksgaard, Copenhagen in *Histocompatibility Testing 1977*, edited by W. Bodmer *et al.*

KNOWLEDGE of the major histocompatibility system of man, the HLA system, has advanced rapidly in recent years. It includes a still increasing number of closely linked loci, each with multiple alleles, controlling cell surface determinants important for humoral and cellular immunity. The biological importance of this genetic region is, furthermore, substantiated by the close association between some of the genes belonging to this region and an increasing number of diseases. It is known that the genetic information for HLA is on chromosome 6 and effectively includes the genes for the complement factors C2, C3, C4 and Bf as well as the erythrocyte systems Rodgers (Rg) and Chido (Ch). The polymorphic enzymes phosphoglucomutase-3 (PGM<sub>3</sub>) and glyoxalase (GLO) are also on chromosome 6 but clearly separated from HLA. Knowledge of the genetic map of chromosome 6 is rapidly increasing, primarily because of the tremendous polymorphism of the HLA system which makes nearly every family informative in segregation analyses.

The rapidly increasing knowledge of the HLA system has depended heavily on the stimulus provided by seven international workshops in histocompatibility between 1964 and 1977.

The main subjects of the seventh workshop were:

(1) Serological investigations of the human Ia (=immune associated) determinants present on B lymphocytes and not on T lymphocytes.

(2) The genetics of these Ia determinants as revealed by family studies, including recombinants, and the relationship between the Ia specificities as detected by serological techniques

and the Dw determinants as revealed by typing in mixed lymphocyte cultures (MLC) using homozygous typing cells (HTC).

(3) Serological investigations of 'old' and new specificities belonging to the HLA-A, B and C loci.

(4) Investigations of a limited number of diseases in order to show whether they were more closely associated with Ia determinants than with HLA-(A and) B antigens and D determinants.

One hundred and fifty laboratories from all over the world participated allowing antisera for the definition of Ia determinants and HLA-A, B and C antigens to be tested against all major racial groups. For family studies data for more than 3,500 family members was included in the workshop: they were all tested with Ia antisera and most with HLA-A,B,C and some with HTCs to reveal Dw determinants. This resulted in a collection of nearly 800 haplotypes and the material included 122 recombinants of whom 42 provided information on Ia specificities.

The Ia specificities were generally found to be closely associated with the HLA-Dw determinants as identified with HTCs, but the Ia specificities as serologically identified on isolated B lymphocytes were generally somewhat 'broader' than the associated HLA-Dw determinants. Furthermore, in extensive family material no confirmed recombination between the Ia and Dw specificities was found. It is therefore still unclear whether the Ia specificities are identical to, or only closely associated with the Dw determinants. Biochemical investigations of the isolated gene products are in progress in various laboratories to resolve this question.

The 42 recombinants studied supported the map sequence of the HLA-loci on chromosome 6 as A, C, B and D.

As usual a WHO nomenclature committee met immediately after the workshop and discussed which specificities could be upgraded to full HLA-status from their previous provisional designation, indicated by a w before the number. HLA-A25 and A26 (splits of HLA-A10) and HLA-B15, B17, B37 and B40 were so upgraded.

Thirteen HLA-B specificities achieved provisional w-status and were designated Bw45 to Bw54. These specificities included 'splits' of previously designated specificities such as B5, B12, Bw21 and Bw22. The old broad specificities, 4a and 4b were named Bw4 and Bw6 as

they were found particularly useful in defining splits of 'broad' HLA-B antigens.

One new C-locus specificity, Cw6 (previously T7), was agreed on, and a total of six Cw-specificities can now be recognised, some of which are well enough defined to qualify for full HLA status. However, it was agreed, for the time being, to continue to designate them Cw1 to Cw6 in order not to risk confusion with the complement (-C-) factors.

The MLC typing allowed identification of five new Dw determinants Dw7 to Dw11, but all the Dw determinants remained provisional (w) as the correlations between some of the typing cells used for identification of the individual determinants were rather low, most probably because of inclusion phenomena and cross reactivity as known from the HLA-A, B and C serology.

The Ia specificities as established by serological assays on B lymphocytes were designated DRw followed by consecutive numbers from 1 to 7. It was clearly shown that these DRw specificities were highly associated with the Dw-related specificities, but it remains for future investigations to show whether they are identical to the Dw determinants.

## HLA and disease

More than 2,500 patients were included in the workshop covering the following diseases: ankylosing spondylitis, multiple sclerosis, juvenile diabetes, psoriasis, active chronic hepatitis, idiopathic haemochromatosis, myasthenia gravis and rheumatoid arthritis and several others, to some of which DRw typing was applied for the first time.

Several of the diseases had previously shown associations with B locus antigens. In some cases the DRw association was roughly the same as the previously noted B locus association, for example, the relative risk for DRw3 in juvenile onset diabetes, myasthenia gravis and active chronic hepatitis was between 2 and 3 which was the level of risk for the antigen B8 with which DRw3 is in strong linkage disequilibrium. In the case of multiple sclerosis, the relative risk for DRw2 of 3.8 was similar to that found for the Dw2 antigen, and is much higher than the risk for B7 previously shown for this disease. The high association of A3 with haemochromatosis was confirmed with a relative risk of 9, this being of particular interest as it is the only disease so far showing a clear cut A locus association. A C locus association was found for psoriasis: Cw6, a newly defined antigen showed a relative risk of 4.3 in psoriasis and explained the previously noted associa-

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tions with B13 and B17, with which Cw6 is in linkage disequilibrium.

Of greatest interest for the Ia serology were the associations found with DRw4 in juvenile onset diabetes and rheumatoid arthritis. In juvenile onset diabetes DRw4 has a similar relative risk to the previously noted B8 and DRw3 and obviously points to a complicated mode of inheritance for susceptibility. The association of DRw4 with rheumatoid arthritis is exciting because it is found in 60–70% of patients, as opposed to about 20% in the normal population. This approaches the frequencies seen for B27 for ankylosing spondylitis patients in early studies. With strict definition of ankylosing spondylitis now, the frequency of B27 approaches 100% and it may be that further studies on rheumatoid arthritis will reveal higher frequencies of DRw4 in some groups of patients. As a general conclusion it was clear that where patients had been Dw typed by MLC, very good agreement was found with the serological DRw typing and that the latter would be a preferable method for the studies of disease association with the HLA region, being simpler and less laborious.

#### Other activities

Special interest was focused on the possible relationship between DRw incompatibilities and results of human transplantations. Some groups reported on B cell cross matches before transplants and concluded that a positive B cell cross match seemed to be irrelevant to transplant survival, but the picture was obscured by the influence of 'cold' B cell active autoantibodies. Post-transplant investigations seemed to support a correlation between development of B cell active antibodies and rejection.

J. Dausset (Paris) and collaborators had DRw-typed 96 haploidentical skin grafts performed on family material 8 years ago. They confirmed their previously reported correlation with HLA-A and B incompatibilities and graft survival, but they also found a highly significant correlation between skin graft survival and the number of DRw incompatibilities. It is clear that further investigations in this area are needed.

Cell mediated lympholysis (CML) testing was the subject of an unofficial working group. The test involves education of cytotoxic lymphocytes (CTLs) in MLC-inducer cultures and subsequent testing against target cells in a chromium-51 release assay.

It has until now been dogma that the serologically defined HLA-A, B, C antigens are the actual targets for destruction in CML, while the influence of HLA-D determinants is unknown. There are, however, now

multiple indications that CTLs either recognise HLA-A, B, C antigens in a way different from that of antibody, or alternatively recognise determinants governed by loci other than HLA-A, B, C. There is even evidence of CML determinants controlled by chromosomal regions other than HLA, for example the male Y-chromosome, although killing through these determinants seems to be restricted by certain HLA antigens.

Thirty CTLs giving results inexplicable by HLA-A, B, C, D antigens have been located and correlated in an international CML workshop. Three tentative CML-defined specificities could be identified, and they may be of allelic genetic origin. Each of the putative CML-traits showed, however, correlations with HLA-antigens.

In conclusion, there may be reasons to believe that at least some CTLs produced *in vitro* recognise separate antigenic determinants, although much more work is needed before this statement can be finally substantiated.

The next workshop will be held in the US in about three years time and will be chaired by P. Terasaki. It will deal mainly with DRw and A, B, C serology, genetics of DRw, disease associations and transplantation. □

## Somatomedins and related growth factors

from M. M. Rechler and S. P. Nissley

A workshop on somatomedins and other growth factors was held at the National Institutes of Health, Bethesda, Maryland, 19–21 September, 1977. The conference was sponsored by the National Institute of Arthritis, Metabolism and Digestive Diseases, National Cancer Institute, National Institute of Child Health and Human Development and the John E. Fogarty International Center. The workshop was organised by M. M. Rechler and S. P. Nissley, NIH.

THE somatomedins are a group of polypeptides which are thought to mediate the effects of growth hormone on skeletal growth (see *News and Views* 267, 308; 1977).

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At the workshop major advances were reported in the chemical characterisation of the various somatomedins. R. Humbel and E. Rinderknecht (University of Zurich) presented the complete amino acid sequence of insulin-like growth factor I (IGF-I), formerly known as NSILA I, one of the somatomedins purified from human plasma. IGF-I is a single chain peptide of molecular weight 7650. Its sequence is homologous with approximately 50% of the A and B chains of insulin; 17 of the 19 invariant residues of insulin are present in IGF-I. The region of IGF-I corresponding to the connecting peptide of proinsulin is shorter than that of human proinsulin (12 residues rather than 35). IGF-I also contains an 8-residue extension beyond the carboxy-terminus of the insulin A chain. Although the location of only one disulphide bond has been identified, the other four half-cystine residues align perfectly with the half-cystine residues in proinsulin. IGF-I shows approximately the same degree of sequence homology with both evolutionarily recent and primitive insulin molecules, leading Humbel and Rinderknecht to propose that IGF-I arose by gene duplication before the evolution of the vertebrates.

The relationship of IGF-I to the other somatomedins isolated from pooled human plasma remains to be determined. Rinderknecht and Humbel have previously reported that more than 70% of the 30 amino terminal amino acid residues are identical in IGF-I and IGF-II. Both IGF-I and II, and somatomedin C have isoelectric points of 8.2–8.6. Unlike IGF-I, somatomedin A is a neutral peptide and possesses only one half-cystine residue (L. Fryklund, AB Kabi, Stockholm). Fryklund also presented the complete amino acid sequence of somatomedin B. Although plasma levels of somatomedin B are growth hormone dependent, somatomedin B differs in chemical composition and biological properties from the other somatomedins. Somatomedin B has no sequence homology with insulin, consistent with its lack of insulin-like activity, but does show homology with protease inhibitors, and preliminary results indicate that somatomedin B has anti-trypsin activity.

A major enigma has been the relationship of the low molecular weight NSILA to NSILP (human serum non-suppressible insulin-like protein) purified by P. Poffenbarger (University of Texas, Galveston). NSILP is a glycoprotein of molecular weight 88,000 composed of two non-identical chains. The Zurich group finds that most of the non-suppressible insulin-like activity in plasma is found in a low molecular weight form following acid treatment



of serum whereas in Poffenbarger's purification scheme which avoids acid exposure, the large molecular weight NSILP accounts for all of the activity. R. Froesch (Zurich) presented the provocative observation that low molecular weight NSILA, combined with its serum binding protein (see below) and chromatographed on Dowex, is eluted as a higher molecular weight form that can no longer be dissociated by acid. Since Dowex chromatography is the first step in Poffenbarger's purification scheme for NSILP, Froesch proposed that NSILP may represent NSILA plus its binding protein.

Somatomedins have also been purified to homogeneity from a rat liver cell line (MSA, multiplication stimulating activity) and from rat serum (rat 'somatomedin'). MSA is a neutral peptide of molecular weight 8700 containing 3-4 half-cystines, carboxy-terminal glycine and a blocked  $\alpha$ -terminus (A. Moses, NIH). Residues 19-38 of rat somatomedin, a basic peptide with three disulphide bridges distantly resemble the A chain of insulin and relaxin (W. Daudet, J. Jacobs, Washington University, St Louis). Rat somatomedin and MSA are clearly different polypeptides since they show no cross reactivity in specific radioimmunoassays (J. Daniels, Washington University, St Louis; P. Nissley, NIH).

### Assays

The cross reactivity of the different peptides is so extensive that no assay yet exists that exclusively measures only one of the somatomedin peptides. Thus when these assays are used to determine 'somatomedin' levels in serum the composite effect of several peptides is being studied; the relative contribution of each somatomedin depends on the assay used.

Results from bioassays and competitive binding assays clearly establish that IGF-I, IGF-II, somatomedin A, MSA and probably somatomedin C constitute a class of closely related but not identical polypeptides. IGF-I, IGF-II, somatomedin A and MSA possess 1/50th-1/500th the activity of insulin in several *in vitro* assays in fat cells (J. Zapf, Zurich). These four purified somatomedins are also potent stimulators of DNA synthesis in chick embryo fibroblasts (Zapf; M. Rechler, National Institutes of Health). Somatomedin A and a preparation containing a mixture of IGF-I and IGF-II are equipotent in stimulating both uridine and sulphate incorporation into chick embryo cartilage; MSA was active but 10-20 times less potent (K. Gibson, Roche Institute of Molecular Biology, Nutley).

Collaborative studies (K. Hall, Karo-

linska Hospital, Stockholm; Rechler; Zapf) revealed extensive cross reactivity between IGF-I, IGF-II, somatomedin A and MSA in competitive binding assays in which  $^{125}$ I-labelled homogeneous somatomedins bound to either the somatomedin receptors of various different cell types, or to a human serum carrier protein for somatomedin.

Autoantibodies against the insulin receptor isolated from a patient with a rare form of insulin resistance, block the binding of  $^{125}$ I-MSA to somatomedin receptor on human skin fibroblasts suggesting that the somatomedin receptors on human fibroblasts share antigenic determinants with insulin receptors (Rechler).

Recently developed radioimmunoassays for the human somatomedins discriminate better among the somatomedin-related peptides than do bioassays and radioreceptor assays. R. Furlanetto and J. Van Wyk (University of North Carolina, Chapel Hill) reported that somatomedin A is approximately 3% as potent as somatomedin C in a somatomedin C radioimmunoassay.  $^{125}$ I-MSA does not bind to the somatomedin C antibody. In a radioimmunoassay utilising IGF-I tracer and an antibody directed against IGF-I, somatomedin A is equipotent with IGF-I whereas IGF-II is 30-50 fold and MSA 500-fold less potent than IGF-I (Zapf). Hall presented preliminary data for a radioimmunoassay utilising  $^{125}$ I-labelled somatomedin A tracer and antibody directed against somatomedin A in which IGF-I is approximately 10-fold more potent and IGF-II 10-fold less potent than somatomedin A. MSA does not cross react.

### Binding proteins

Somatomedins are distinctive among polypeptide hormones in that they are associated with specific binding proteins in the circulation. There was general agreement that when radio-labelled IGF-I, IGF-II, somatomedin A or somatomedin C are incubated with human serum and analysed by gel chromatography, all ligands show a similar binding profile (Zapf; Fryklund; Van Wyk; D. Schall, University of Colorado, Denver; R. Hintz, Stanford University). Most of the specifically bound tracer elutes near the position of albumin with a minority of the tracer being bound to a larger molecular weight protein (125,000-200,000). There was also general agreement that a 60,000 binding protein could be generated by acid treatment of the larger binding protein suggesting that the larger binding protein is an oligomer of the smaller one. In rats, both Moses and Zapf presented data showing that  $^{125}$ I-MSA and  $^{125}$ I-NSILA bind to large

molecular weight proteins in normal rat serum, and that both the binding profile on Sephadex G-200 and the binding capacity are growth hormone dependent. C. Meuli (University of Zurich), studying glucose uptake in rat heart perfused through the coronary arteries, found that addition of carrier protein to the perfusate abolishes the stimulation by NSILA. Meuli attributed this inhibition to an inability of the carrier protein-NSILA complex to diffuse readily out of the capillary bed. It is not known whether the *in vitro* anabolic actions of somatomedin are also inhibited by the binding protein.

### Other growth factors

In a discussion of some of the non-somatomedin polypeptide growth factors R. Andre (Washington University, St Louis) presented data supporting a model of nerve growth factor (NGF) action in which NGF initially binds to cell surface receptors, is internalised by pinocytosis and ultimately binds to nuclear receptors. K. Lembach (Vanderbilt University, Nashville) described studies showing inhibition of  $^{125}$ I-EGF (epidermal growth factor) binding to human skin fibroblasts by lectins specific for mannose, galactose and N-acetyl galactosamine residues, suggesting that the EGF receptor is a glycolipid or glycoprotein containing those residues. The carbohydrate nature of the EGF receptor was supported by data of R. Pratt and I. Pastan (NIH) showing that a Balb/c 3T3 mutant (AD6) which exhibits impaired biosynthesis of complex carbohydrates and glycoproteins due to a block in acetylation of Glc N-6-P, also shows decreased binding of EGF to cell surface receptors. AD6 cells grown in the presence of GlcNAc increased their binding of  $^{125}$ I-EGF by 50%. D. Gospodarowicz (University of California, San Francisco) showed the complete amino acid sequence of brain fibroblast growth factor (FGF) and described the pathway by which a 169 residue inactive precursor molecule is converted into biologically active FGF consisting of residues 44-153. Gospodarowicz also reported that the entire FGF sequence is contained within the sequence of brain myelin basic protein. H. Antoniades (Harvard University, Boston) described the purification from serum of a heat stable cationic (pI 9.7) polypeptide of molecular weight 13,000. By radioimmunoassay this factor is present in small quantities in serum derived from platelet-poor plasma. A. Vogel and R. Ross (University of Washington, Seattle) reported the partial purification of a similar factor from platelets which they propose is the principal mitogen in blood serum responsible for the stimulation of DNA synthesis in tissue culture. □

# review articles

## The multi-disciplinary role of 'pion factories'

J. D. Davies\*, C. J. Batty† & K. Green†

*The multi-disciplinary role of intermediate energy proton accelerators in pure and applied nuclear physics is discussed with particular reference to the experimental programmes at LAMPF (Los Alamos Meson Physics Facility) and SIN (Swiss Institute for Nuclear Research, Zurich).*

INTERMEDIATE energies, in the context of this discussion, refer to proton accelerator energies above the threshold for pi-meson production but below that for K-mesons, say 400 MeV up to 1 GeV. That is above the energies of the Van de Graff machines used for nuclear structure studies but below the multi-GeV proton synchrotrons used in high energy elementary particle physics. About 1960, experiments in this energy range were reaching the limits of technique with the then available intensities of the variable frequency synchrocyclotrons and so interest shifted to the new, higher energy proton synchrotrons where high energy particle physics has thrived. However, a very rich field remained, awaiting a new generation of proton accelerators with intensity increases of 3 to 4 orders of magnitude; the so called 'pion factories'. These machines have now been operational for a few years and it is an interesting time to review their role as multi-disciplinary facilities. Relevant parameters of these new accelerators are listed in Table 1 together with those of some older but recently upgraded machines. Duty cycle is an important but often neglected parameter in that it governs the type of experiment for which the machine is best suited.

The proton beams from these accelerators can be used in their own right but in general other particles such as pions, muons, protons, neutrons, electrons, photons and neutrinos are generated through proton interactions in massive targets. Some particle properties are listed in Table 2. Typical particle fluxes to be obtained from 100  $\mu$ A of proton beam are

$1 \times 10^{16}$ spallation neutrons at $\sim 1$ MeV	
$1 \times 10^9 \pi^+/\text{s}$	} in the energy range 100–600 MeV
$1 \times 10^8 \pi^-/\text{s}$	
$1 \times 10^7$ stopping pions per s	
$6 \times 10^6$ stopping muons per s	
$3 \times 10^{14}$ neutrinos per s at source	

The exact fluxes depend on the geometry of the beam concerned and in particular muon beams, which arise from pion decay, are very dependent on the exact layout. The highest intensity muon beam is at SIN<sup>1</sup> where a superconducting solenoid system collects and transports  $3 \times 10^7 \mu^- \text{s}^{-1}$  at 125 MeV/c.

Such beams of particles are now being used for a wide range of experimental investigations, from the pure science aspects of nuclear and particle physics through their use as diagnostic tools in the applied fields of  $\mu$ SR and mesic X-rays in condensed matter research to the directly practical fields of isotope production and

medical therapy. These fields will now be discussed in more detail with particular reference to characteristic experiments which are being carried out at the SIN and LAMPF meson factories.

### Particle and nuclear physics

Particle and nuclear physics continue to play a central part in the programme of experiments although continually challenged by the increasingly important part played by the applied nature of much of the research. The weak interaction is being extensively studied at SIN and LAMPF and provides an ideal example of how the study of rare processes using the high fluxes from pion factories can successfully complement experiments at the high-energy machines which explore the sub-structure of 'elementary' particles. The conventional form for the weak interaction, with only vector and axial vector charged currents ( $\mathbf{V}-\mathbf{A}$ ), has been shattered in experiments at high energies involving neutrinos and has opened up once again the whole question of the form of the weak interaction and the associated conservation laws. Muon decay experiments are free from strong interaction effects but are insufficient alone to specify the charged current weak interaction Hamiltonian. One needs to assume  $m_{\nu_e} = m_{\nu_\mu} = 0$  and to look for indirect evidence from hadronic decays.

Limits on the neutrino mass are obtained by measuring momenta in the decay  $\pi \rightarrow \mu + \nu_\mu$ ; presently a SIN experiment has set an upper limit<sup>2</sup> of 0.5 MeV for the mass but both SIN and LAMPF expect eventually to reach 0.2 MeV. Possible scalar and tensor contributions can be studied through the electron polarisation from muon decay. Limits will be reduced by a factor of 5 at SIN in experiments now under way.

Zero mass for the neutrino forces them to have 100% polarisation and the observed negative helicity accords with the charged current  $\mathbf{V}-\mathbf{A}$  Hamiltonian. The zero spin of the pion then forces its decay muon, or electron, to have the same, but unnatural, helicity and the decay can only proceed since the charged lepton mass is non-zero. The ( $\mathbf{V}-\mathbf{A}$ ) form and electron-muon universality are sufficient to predict<sup>3</sup>

$$R = \frac{\pi^+ \rightarrow e^+ \nu_e}{\pi^+ \rightarrow \mu^+ \nu_\mu} = 1.233 \times 10^{-4}$$

The accuracy of the present experimental value<sup>3</sup> of  $R = (1.274 \pm 0.024) \times 10^{-4}$  will soon be improved by experiments at LAMPF and TRIUMF. Departure of experiment from theoretical prediction would also occur for example if a heavy lepton (L), whose existence has recently been indicated in high-energy experiments was coupled only with the electron neutrino. Furthermore if the heavy lepton is mixed with the muon and electron then the decay  $\mu^\pm \rightarrow e^\pm \gamma$  could occur at a rate  $10^{-9}$  to  $10^{-10}$  of the normal decay. Such a decay, forbidden by separate, additive lepton conservation laws would be of fundamental significance and experiments are in progress at all the meson

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Table 1 Meson facilities

Facility	Accelerator type	Country	Range of proton energy (MeV)	Beam intensity ( $\mu\text{A}$ )		Duty cycle
				Planned	Operated at	
LAMPF	Linear	USA	600–800	1000	100	$6 \times 10^{-2}$ with 0.5 ns per 5 ns
SIN	Cyclotron	Switzerland	590	100	100	1.0 with 1 ns per 20 ns
TRIUMF	Cyclotron	Canada	180–520	100	50	1.0 with 1 ns per 42 ns
SREL	Synchro-cyclotron	USA	600	1–2	1–2	0.5
DUBNA	Synchro-cyclotron	USSR	680	25–50	2	0.5
CERN	Synchro-cyclotron	Switzerland	600	10	2	0.5

factories to search for this decay. The published limit<sup>4</sup>

$$B = \frac{(\mu^+ \rightarrow e^+ \gamma)}{(\mu^+ \rightarrow e^+ \nu \bar{\nu})} < 2.2 \times 10^{-8}$$

has already been reached at SIN and TRIUMF and should be considerably improved in the coming months by experiments at all three laboratories.

The high intensities of the pion factories are now sufficient to give good counting rates in high-resolution spectrometers in nuclear scattering experiments. Until now complex nucleus scattering experiments were restricted to well separated final states such as the ground state of  $^{12}\text{C}$  with its first excited level at 4.4 MeV. The resolution is now sufficiently good to determine the energy state of many recoil nuclei. LAMPF are at present commissioning high-resolution spectrometers for both pions and protons.

At SIN, elastic and inelastic  $\pi^+$  scattering from  $^{12}\text{C}$  and the double charge exchange reaction  $^{18}\text{O}(\pi^+, \pi^-)^{18}\text{Ne}$  have been measured<sup>5</sup>; in the latter they were able to resolve the ground state and 1.89 MeV first excited states, a reaction first reported<sup>6</sup> from LAMPF in an experiment where a resolution of 4 MeV was insufficient to separate the states. Double charge exchange can now become a powerful tool in nuclear structure research, identifying  $\Delta T_z = 2$  isobaric analogue states and giving exciting prospects for looking at proton rich nuclei.

Also at SIN is a high resolution pair spectrometer with large acceptance. This measures the energies of  $\gamma$ -rays coming from radiative pion capture  $N(A, Z)(\pi^-, \gamma)N(A, Z-1)$ , which comprises  $\sim 2\%$  of all captures, and enables studies to be made of the energy levels of the final unstable nucleus<sup>7</sup>.

At LAMPF there has been considerable work<sup>8</sup> on low-energy elastic  $\pi^+$  scattering from selected nuclei, which conclusively demonstrated the importance of both nuclear structure and pion dynamics, and their nucleon–nucleon programme is such that it is only a matter of time until a complete set of experiments will have been done on elastic scattering, measurements of the spin-dependent parameters, and all inelastic channels.

## Exotic atoms

A negative muon or pion will be slowed down in matter in a time of the order of  $10^{-10}$  s, which is considerably smaller than the particle's lifetime. Being negatively charged it can be captured into an atomic orbit about the positively charged nucleus. The atomic levels for a given set of quantum numbers are increased in energy by the ratio of the particle mass to electron mass compared with the corresponding electron orbits whilst the classical radii of the atomic orbits are decreased by the same factor. This 'exotic' atom will become de-excited to orbits of lower principal quantum number ( $n$ ), first by Auger transitions and later by X-ray emission. A muon eventually reaches the  $n = 1$  orbit where it either decays or is captured by the nucleus. Because of the strong interaction a pion will be captured by the nucleus as soon as it reaches a state of low enough angular momentum where the pion wavefunction has a significant overlap with the nucleus. Measurement of these X-ray spectra can yield a wealth of information ranging through studies of quantum electro-dynamics (QED) and nuclear structure to studies of atomic cascades and applications to chemical analysis.

Precise measurements of X-ray energies for muonic atoms made 3 to 4 years ago seemed to indicate a discrepancy<sup>9</sup> with values

calculated using QED. Improved measurements made more recently<sup>10</sup> together with further refined evaluation<sup>11</sup> of the 'vacuum polarisation' terms in the QED calculations now give very satisfactory agreement. Measurements of X-ray energies for muonic atoms are still of great interest, however, as the values for the lower  $n$  states can give valuable information on the nuclear charge distribution. Experimenters at CERN, LAMPF and SIN are collaborating in very precise measurements of muon transitions in  $A \sim 40$  to  $A \sim 95$  isotopes<sup>12</sup>. This is providing a systematic study of nuclear charge distributions and its changes with  $Z$  and  $N$ . Measuring hyperfine splittings gives information on nuclear electric multipole moments and work of this type<sup>13</sup> is being done at LAMPF for the osmium and platinum isotopes.

In the field of applications the use of muonic X-rays for *in-situ* analysis of bulk material composition is being investigated<sup>14,15</sup> at LAMPF. The method is non-destructive and by varying the beam parameters it is possible to investigate the distribution of elements through the volume of the object. The method has the advantage that it can be used for any element and is of particular interest for light elements where methods such as neutron activation are not suitable. In the case of medium and high  $Z$  elements it is sometimes possible to distinguish between isotopes of the same element. Typical sensitivities are of the order of a few parts per thousand.

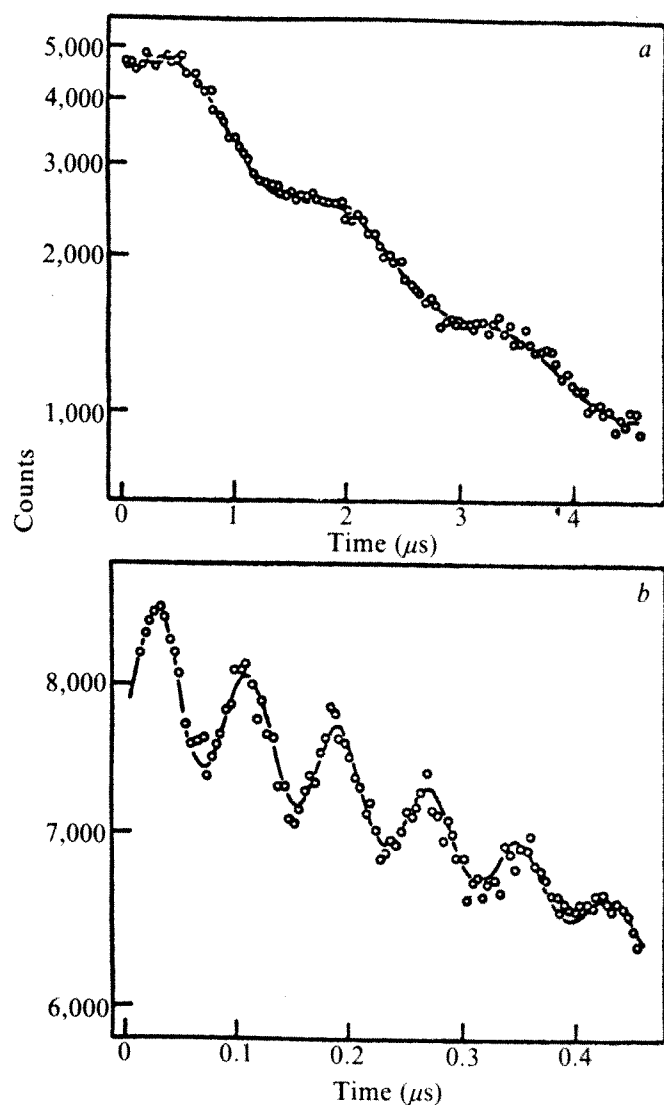
So far we have assumed that the relative concentrations of different elements can be deduced from measurements of the relative X-ray intensities. However, it is now known that the chemical and physical form can influence the relative intensities of X rays from exotic atoms of a particular element. This is because the particle is initially captured into an atomic state of large radius which can be affected by the atomic and molecular electron clouds and hence by the chemical and physical form of the material. Thus studies of mesic X-ray spectra could yield considerable information about the structure of the outer electron shells and there are extensive programmes of work to study these phenomena at several laboratories. At present the subject is an interesting branch of atomic physics but the improved and systematic experiments now in progress will require detailed comparisons with realistic theoretical models before exotic atom chemistry can become a serious and reliable tool for chemical and solid state studies.

What additional information is obtained by studies of pionic X-rays? As already mentioned the strong interaction with the nucleus causes the pions to be captured if their wavefunction has a significant overlap with the nucleus. As a result the lowest observable level will be broadened and shifted in energy from the QED values and measurements of these effects can be a powerful tool<sup>17</sup> for investigations of pion-nucleus interactions. This is a topic of particular significance because the pion-two-nucleon interaction is expected to dominate. Capture on single nucleons

Table 2 Particle properties

Particle type	Mass (MeV)	Spin ( $\hbar$ )	Lifetime (s)	Principal decay mode
Neutron	939.6	$\frac{1}{2}$	918	$p + e^- + \bar{\nu}$
Proton	938.3	$\frac{1}{2}$	Stable	—
Pion	139.6	0	$2.6 \times 10^{-8}$	$\mu + \nu$
Muon	105.7	$\frac{1}{2}$	$2.2 \times 10^{-6}$	$e + \nu + \bar{\nu}$
Electron	0.511	$\frac{1}{2}$	Stable	—
Neutrino	$\sim 0$	$\frac{1}{2}$	Stable	—





**Fig. 1** Muon precession signal in paramagnetic (a) and ferromagnetic (b) Ni. This figure is based on one in the article "On the application of polarised muons in solid state physics" by A. Schenek<sup>32</sup>.

bound in a nucleus is strongly suppressed by energy and momentum conservation.

A very wide range of measurements of shifts and widths of pionic X-ray transitions are now available. The high intensity stopped pion beams make measurements with separated isotope targets possible so enabling comparisons to be made between different isotopes of the same element.

### Biomedical applications

There has been considerable development work at many accelerators on the use of negative pions for the treatment of cancer. Clinical studies with human patients started in 1974 at LAMPF<sup>18</sup> and SIN<sup>19</sup> are building a large medical centre with treatment of patients planned for 1979. Negative pions have physical and biological properties that could lead to significant improvements in radiation therapy which presently is used for more than half of cancer patients.

A negative pion can be made to pass through normal tissue with low ionisation dose and then to stop in a small well defined volume with high dose. This high dose results from both the increase in ionisation rate as the pion slows down and the short range fragments coming from the stopped pions which annihilate on a nucleus: a significant fraction of the pions rest mass of 140 MeV goes into producing heavy nuclear ions. Anoxic (oxygen deficient) cells commonly found in tumours have an intrinsic resistance to damage by radiation but which can be overcome by high linear

energy transfer (LET). The high ionisation rates of stopping pions and nuclear fragments may help to increase their relative biological efficiency (RBE).

Several laboratories have dedicated biomedical pion beams with intensive and detailed programmes designed to lead to the treatment of patients. Starting with simple systems, radiobiologists are investigating the claims for high LET, the effects on normal tissue and genetic damage. At LAMPF, treatment of selected human patients<sup>18</sup> began in 1974 and has now included tumours in skin, subcutaneous tissue, muscle, oral cavity, chest wall and lung. Measurements on the pion beams and the stray radiations, physical dose in different tissues and equivalent dose measurements with biological dosimeters are some of the physical and biological dosimetry studies being made.

The well known EMI X-ray scanner provides density reconstruction in axial tomography of tissues for medical diagnosis. At LAMPF an experiment has started using the energy loss of a proton passing through a specimen to determine the density projections: computer simulation has suggested that 0.4% density resolution can be obtained with a proton dose several times smaller than from X rays.

### Isotope production

When a complex nucleus receives energy from a 600 MeV proton or from a pion or muon, it can fragment, and many particles are given off—alpha particles, neutrons, protons and spallation nuclei. Several systematic studies have started, particularly at LAMPF: often the initial reason is pure nuclear physics but frequently applications soon follow.

Of considerable interest are the nuclear and atomic properties of proton- and neutron-rich nuclei far from the mass stability curve that are produced in (p, p Yn) and (p, Xp n) reactions. In studies of very short lived nuclei, the on-line mass isotope separator of the ISOLDE facility<sup>20</sup> at CERN has a major role. The subject also goes hand-in-hand with isotope production for medical and industrial use.

Reactors make neutron-rich products cheaply whilst compact cyclotrons make neutron-deficient isotopes, via (p, Yn) reactions, (where Y = 1, 2, 3) which are relatively free of contaminants. The two sources and types of isotope essentially do not compete. For nuclear medicine application neutron-deficient isotopes will almost always be preferable since they generally decay by electron capture. Where positrons are emitted, a very attractive form of radiography becomes available with the almost back-to-back annihilation  $\gamma$  rays providing a characteristic signature.

For higher energy protons both production yields and Y increase whilst (p, Xp Yn) reactions also become significant. However, this is a nonspecific reaction with many neutron-deficient isotopes being made but specific decay routes can often overcome this disadvantage. Many useful isotopes can only be made from 800 MeV protons and many attractive isotopes are more easily and cheaply made. For instance<sup>21</sup>, at LAMPF, protons on molybdenum yield many useful isotopes of zirconium, yttrium and strontium. At SIN, they are investigating<sup>22</sup> the medically important <sup>123</sup>I.

### Radiation damage and neutron production

The copious production of low-energy neutrons and spallation products in 800 MeV proton interactions allows the use of these machines for fundamental studies on radiation damage at a high rate. Such damage is a major engineering problem in fission and fusion reactors, especially in the first walls of a possible fusion reactor. Heavy ions are the usual investigatory tool but only for the surface. Through the spallation process a proton beam can be effectively a source of heavy ions throughout the body of the material studied.

So far there have been extensive calculations<sup>23</sup> and one experiment<sup>24,25</sup> on aluminium samples irradiated to a damage level of a few displacements per atom (d.p.a.) which were compared with fission neutron damage of Al to the same d.p.a. The following exciting conclusions were reached. 800 MeV proton bombardment simulates fission and fusion neutron irradiation but

with a much lower heat per d.p.a. so that high current densities or damage rates are possible. Similar void densities for a given d.p.a. were obtained with proton and fission neutron irradiation although the former produces 1,000 times as much He. This is a very significant result since there had been speculation that the high He production per d.p.a. of fusion neutrons compared with those from fission could give a corresponding increase in the void nucleation rate. Additionally, there was no unexpected proton damage although a wide range of impurity atoms was produced.

Neutrons are numerically the greatest product in 800 MeV proton interactions. Each proton can produce 24 neutrons from a tungsten target or 40 from one of uranium; in some sense there are more neutrons from spallation than from fission. In Canada there is extensive interest in using spallation neutrons from intermediate energy accelerators for fertile to fissile conversion. Radiative neutron capture in  $^{238}\text{U}$  and  $^{232}\text{Th}$  leading through  $\beta$  decay to  $^{239}\text{Pu}$  and  $^{233}\text{U}$  are being studied at TRIUMF in the FERFICON<sup>26</sup> project.

## Muon spin rotation

Muon spin rotation ( $\mu\text{SR}$ ), so named because of its close analogy with nuclear magnetic resonance (NMR) and electron spin resonance (ESR), performs many functions of these well-tried techniques in the study of condensed matter but in addition has many advantages of its own<sup>27</sup>. The method originated in experiments on the  $(g-2)$  and magnetic moment of the muon. The former has tested quantum electrodynamics to better than 1 part in  $10^8$  and demonstrates that muons behave as heavy electrons. At SIN they are determining the muon magnetic moment to 1 part per million by measuring its Larmor precessional frequency in a known magnetic field.

The principle of  $\mu\text{SR}$  is to determine the Larmor precession frequency and the relaxation time of polarised muons by observing the muon decay asymmetry as a function of time. This field pioneered in the 1970s by the Berkeley group at the 700 MeV cyclotron is an ideal example of the cross-fertilisation of the ideas between nuclear particle physicists and condensed matter research. There are two kinds of muon spin rotation,  $\mu^+$  SR and  $\mu^-$  SR; in the former the muon behaves as a light proton and in the latter it behaves as a heavy electron. In the former case the  $\mu^+$  diffuses interstitially through the material whilst for the latter the  $\mu^-$  will be bound in the ground state of a muonic atom and hence located close to the nucleus.

A typical experiment of this type involves slowing down and implanting in the sample one of these muons whose spin direction is well defined. The process of thermalising the muons occurs on a short enough time scale ( $\sim 10^{-10}\text{s}$ ) such that the full spin polarisation of the muon is preserved. The muon spin will then precess about the local magnetic field at a frequency determined by this field and the muon magnetic moment until such time as it decays  $\mu^+ \rightarrow e^+ + \nu_e + \bar{\nu}_\mu$ . The probability for the muon to decay at a given time is a constant and leads to a characteristic exponential decay curve for the muon with a time constant of  $2.2\mu\text{s}$ . The positron ( $e^+$ ) is emitted in the decay preferentially along the muon spin direction so that observation of this positron implies the direction of the muon spin after a time ( $t$ ). Observations of many such muons, all decaying at different times ( $t$ ) leads to a curve of the type shown in Fig. 1. As can be seen the characteristic exponential decay of the muon has superimposed upon it an oscillation whose frequency is dependent upon the local magnetic field seen by the muon. The difference between paramagnetic and ferromagnetic nickel is dramatic and obvious.

The advantages to be gained in using  $\mu\text{SR}$  as compared with other techniques like NMR, ion implantation, Mossbauer effects or even neutron diffraction in solid state physics are based on the fact that the muon is point-like with no structure or significant electromagnetic perturbations; its spin is  $\frac{1}{2}\hbar$  and therefore it has no quadrupole moment. The fact that  $10^6$  to  $10^7$  muons are sufficient to give an adequate signal and with only a few muons present in the sample at any one time means that cross talk and disruption or radiation damage to the host is minimal.

Uses to which this technique can be put are many and varied but include studies of magnetic effects<sup>28</sup>, diffusion processes and of impurity centres in semiconductors and insulators. For example at SIN positive muons are being used to look at diffusion effects in metals as a function of temperature<sup>29</sup> and to study atomic defects. In the latter case the muon has considerable advantages over positrons, which can also be used. Because of its greater mass the muon can be used to detect traps with a potential too weak to find a positron and in certain cases absolute defect concentrations can be measured. A similar programme of experiments has been proposed at LAMPF.

Muonium ( $\mu^+e^-$ ) is formed in the slowing down of positive muons in non-metallic materials, particularly in liquids. The existence of these thermal sources of relatively stable bound muons allows chemical effects to be studied. For example at SIN comparisons<sup>30</sup> are being made between muonium (mass  $\sim 1/9 m_H$ ) reaction rates with those for the corresponding H atom rates. Previous studies of isotope effects were limited by the 1:3 mass ratio between  $^1\text{H}$  and  $^3\text{H}$ .

## Neutron sources

We have tried to show in this review that a high-intensity intermediate energy proton accelerator can not only be used for the traditional studies in nuclear and elementary particle physics but can also support a full, wide-ranging and rewarding multi-disciplinary programme of experiments. The meson factories at LAMPF, SIN and at TRIUMF in Canada, all conceived with the pure research aspects of nuclear and particle physics in mind, are now assuming an increasingly important role in these applied aspects. One such topic which is becoming important at LAMPF and at TRIUMF and which is being considered around the world for future generations of meson factories is the use of intermediate energy accelerators as primary sources for the generation of very high neutron flux densities, greater than those available from conventional nuclear fission reactions.

The use of neutrons to study the structure of matter is now a well established technique and Europe, with Britain well to the fore, has a wide programme of work in this field using reactor facilities both nationally and at the French, German and British Institute Laue-Langevin in Grenoble, France. The condensed matter research community in their striving for ever more intense and versatile sources have now realised that a proton accelerator could be the basis of the next generation of facilities for this type of work. We have already mentioned that through the spallation process, a 800 MeV proton can give up to 40 neutrons when it strikes a uranium target. This, together with the fact that the proton beam can be pulsed, so enabling time-of-flight methods to be used, could give a neutron source with between one and two orders of magnitude improvement over that currently available from the best reactors.

Such a proton based source has been proposed at the Argonne laboratory in the USA, in the USSR, whilst in Britain the Science Research Council has just received approval for a similar project to be built at the Rutherford Laboratory. This latter project<sup>31</sup> will use much of the present NIMROD accelerator now engaged in high-energy physics research and which is due to be closed down in mid 1978. The new facility in its basic form will provide 200  $\mu\text{A}$  of 800 MeV protons in 53 pulses per s each of 200 ns duration. In principle such a machine could also be used for experiments other than neutron scattering. It is to be hoped that these new machines will embody flexible duty cycles as part of their design criteria and enable the full range of multi-disciplinary uses of an intermediate energy accelerator to be carried out at the one facility: the cross fertilisation of ideas being of benefit to the whole community. The programmes of research at SIN, LAMPF and TRIUMF have shown the way for the community to benefit from the expensive and sometimes remote world of high energy particle and nuclear physics.

Reports on proposed experiments and work in progress are given in Annual Reports from the SIN and TRIUMF laboratories and in Newsletters from LAMPF and SIN. We have used these sources extensively in the preparation of this article.



- <sup>1</sup> SIN Newsletter 6, 4 (1976).
- <sup>2</sup> Daum, M. *et al.* *Phys. Lett.* **60B**, 380–384 (1976); *SIN Newsletter* **8**, 26 (1977).
- <sup>3</sup> Bryman, D. & Picciotto, C. *Phys. Rev.* **D11**, 1337 (1975).
- <sup>4</sup> Parker, S., Anderson, H. L. & Rey, C. *Phys. Rev.* **133B**, 768–778 (1964).
- <sup>5</sup> SIN Newsletter **8**, 22–23 (1977).
- <sup>6</sup> Marks, T. *et al.* *Phys. Rev. Lett.* **38**, 149–152 (1977).
- <sup>7</sup> Alder, J. C. *et al.* *Proceedings Conference on Meson-Nuclear Physics*, A.I.P. Conference Proceedings No. 33, 624–625 (1976).
- <sup>8</sup> Amann, J. F. *et al.* *Phys. Rev. Lett.* **35**, 426–429 (1975).
- <sup>9</sup> Dixit, M. S. *et al.* *Phys. Rev. Lett.* **27**, 878–881 (1971).
- <sup>10</sup> Tauscher, L. *et al.* *Phys. Rev. Lett.* **35**, 410–412 (1975).
- <sup>11</sup> Watson, P. J. S. & Sundarsen, M. K. *Can. J. Phys.* **52**, 2037–2059 (1974).
- <sup>12</sup> Shera, E. B. *et al.* *Phys. Rev.* **C14**, 731–747 (1976).
- <sup>13</sup> Los Alamos Report LA-6553-PR, 74–75 (1976).
- <sup>14</sup> LAMPF Users Newsletter **9**, No. 1, 59–61 (1977).
- <sup>15</sup> Hutson, R. L. *Los Alamos Report LA-5867-MS* (1975).
- <sup>16</sup> Gershtein, S. S. & Ponomarev, L. I. *Part III Muon Physics* (eds Hughes, V. W. & Wu, C. S.) 142–233 (Academic, New York and London, 1975).
- <sup>17</sup> Backenstoss, G. *A. Rev. nucl. Sci.* **20**, 467–508 (1970).
- <sup>18</sup> Kligerman, M. M. *Proceedings of the International Conference on Cyclotrons and their Applications* 419–426 (Birkhauser, Basel, 1975).
- <sup>19</sup> *Proceedings SIN Pion therapy workshop* (unpublished).
- <sup>20</sup> *CERN Courier* **16**, 256 (1976).
- <sup>21</sup> Grant, P. M., Kahn, M. & O'Brien, H. A. *Jour. Inorg. Nucl. Chem.* **37**, 413–417 (1975).
- <sup>22</sup> Hegedus, F. & Peck, N. F. *SIN Physics Report No. 171* (1976).
- <sup>23</sup> Coulter, C. A., Parkin, D. M. & Green, W. V. *J. nucl. Materials* **67**, 140–184 (1977).
- <sup>24</sup> *Lamp users newsletter* **9**, 61–63 (1977).
- <sup>25</sup> Los Alamos Report LA-6678-RR, 96–97 (1977).
- <sup>26</sup> *TRIUMF Annual Report* 45 (1975).
- <sup>27</sup> Brewer, J. H., Crowe, K. M., Gyax, F. N. & Schenk, A. *Part III Muon Physics* (eds Hughes, V. W. & Wu, C. S.) 3–139 (Academic, New York & London, 1975).
- <sup>28</sup> Graf, H. *et al.* *Phys. Rev. Lett.* **47**, 11–14 (1977).
- <sup>29</sup> Camani, M. *et al.* *SIN Newsletter* No. 7 23–23 (1976).
- <sup>30</sup> Percival, P. W. *et al.* *Chem. phys. Lett.* **47**, 11–14 (1977).
- <sup>31</sup> *Rutherford Laboratory Report RL-77-064 C* (1977).
- <sup>32</sup> Schenk, A. in *Nuclear and Particle Physics at Intermediate Energies* (ed. Warren, J. B.) (Plenum, New York, 1976).

## Basic and applied research at the TRIUMF meson factory

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*The TRIUMF 520 MeV H<sup>-</sup> cyclotron produces intense beams of protons, pions and muons supporting basic research in nuclear, particle and solid-state physics, nuclear chemistry and biomedicine, and applied research in electromagnetic breeding of nuclear fuel, proton radiography, radioisotope production and cancer treatment.*

'MESON factory' is the term coined to describe intermediate-energy accelerators designed as prolific sources of pions and muons. So far three capable of delivering proton currents larger than 100  $\mu\text{A}$  have come into operation<sup>1–3</sup>: LAMPF, an 800 MeV proton linear accelerator at Los Alamos, New Mexico (1972); SIN, a 590 MeV proton cyclotron, near Zürich (1973); and TRIUMF, a 520 MeV H<sup>-</sup> cyclotron, in Vancouver (1974). These facilities have proved to be of multidisciplinary interest. Nuclear physicists and chemists can study the interactions of the nucleus either with pions, the particles mainly responsible for holding protons and neutrons together, or with the nucleons themselves, at energies where their de Broglie wavelength is much smaller than a nuclear diameter. Particle physicists can study the properties of nucleons, pions and muons and their interactions among themselves. Solid state physicists can use muons as novel probes for determining magnetic fields in crystal lattices, and neutrons for scattering and diffraction studies of molecular structure. Chemists can use muonium ( $\mu^+ e^-$ ) atoms in the study of chemical reactions in gases. Biomedical scientists can study the effects of the various radiations on living cells. The meson factories also have worthwhile practical applications, particularly in medicine. The localised dose delivered by negative pions holds out the hope for improved treatment of deep-seated cancers. Proton-induced spallation reactions can be used to produce useful proton-rich radioisotopes and to convert fertile to fissile nuclear fuel. Protons can also be used for high resolution, low dose, radiography.

TRIUMF is a cooperative project of the University of Alberta, the University of British Columbia, Simon Fraser University and the University of Victoria—the acronym having been too precious to drop when the original three universities were joined by a fourth. The land, buildings and some administrative services have been provided by the universities; the accelerator and experimental equipment were funded by the Atomic Energy Control Board of Canada from 1968 to March 1976, when responsibility was transferred to the National Research Council of Canada.

### The cyclotron and particle beams

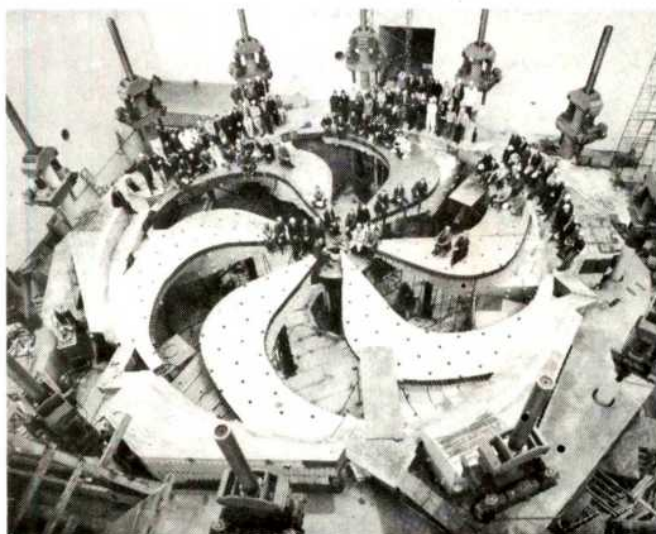
The design of TRIUMF<sup>3</sup> is based on a proposal<sup>4</sup> by J. R. Richardson (director 1971–76) for a meson factory based on a sector-focusing cyclotron accelerating H<sup>-</sup> ions. The chief advan-

tages of this design are (1) continuous, rather than pulsed, beams are produced with modulation only at the RF frequency; (2) the extracted beam energy is continuously variable from 180 to 520 MeV; (3) two or more beams can be extracted simultaneously at independent energies and intensities.

The first feature is not only advantageous for the coincidence detection of nuclear reaction products, but is also the essential characteristic making possible the acceleration of 100  $\mu\text{A}$  beams, rather than the 1  $\mu\text{A}$  typical of the (pulsed) synchrocyclotrons previously available in this energy range. Continuous operation at a fixed frequency is made possible by the division of the magnet into sectors (Fig. 1). These provide periodic focusing forces on the internal beam, thus compensating the vertical defocusing associated with a magnetic field in which the ion orbit time is the same at all energies.

The unique feature of the TRIUMF design, which is crucial to obtaining multiple beams and variable energy, is the acceleration of H<sup>-</sup> ions. Extraction, a notoriously difficult and inefficient

**Fig. 1** The TRIUMF staff assembled on the six lower sectors of the cyclotron magnet, Spring 1972. The 12 columns carry jacks for lifting the entire upper half of the magnet, weighing 2,000 tons.



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Table 1 Beam properties

Beam line	Particles	Polarisation	Energy (MeV)	Momentum spread	Intensity	Spot size (cm × cm)
1/4	p	0	180–520	0.2%	100 $\mu$ A	0.3 × 1.4
1/4	p	70–80%	180–520	0.2%	120 nA	0.3 × 1.4
4A	n	40–75%	(160)–500	(1%)	$6 \times 10^7 \text{ s}^{-1}$	7 × 9
M8	$\pi^+$		20–120	1.5–15%	$\leq 10 \times 10^8 \text{ s}^{-1}$	1 × 2
M9	$\pi^+$		15–65	2–15%	$\leq 3 \times 10^8 \text{ s}^{-1}$	10 × 10
M9	$\pi^+$		stopping	2–15%	$5 \times 10^7 (\text{s-g-cm}^{-2})^{-1}$	3 × 10
M9	$\mu^+$	60%	20–75	2–15%	$\leq 10^7 \text{ s}^{-1}$	
M9	$\mu^+$	100%	4.1	2–15%	$2 \times 10^6 \text{ s}^{-1}$	3 × 10
M20	$\mu^+$	100%	4.1	5%	$5 \times 10^5 \text{ s}^{-1}$	5 × 5
M20	$\mu^+$	60%	90	5%	$10^6 \text{ s}^{-1}$	6 × 6
M11	$\pi^+$		(30–350)	(0.1%)	$(\leq 8 \times 10^8 \text{ s}^{-1})$	10 × 10 (1 × 1)

Intensities of secondary beams are quoted for the proton currents listed.  $\pi^-$  and  $\mu^-$  beams have the same properties as  $\pi^+$  and  $\mu^+$  beams, except that their intensities are a factor  $\sim 5$  lower. Figures in parentheses represent theoretical estimates.

process for circular accelerators, is made trivial by passing the negative ions through a thin carbon or aluminium foil<sup>5</sup>, stripping the two electrons away and leaving positively-charged protons, which then curve away from the cyclotron. In the case of TRIUMF two foils are used, 180° apart in azimuth, to provide two external beams, and each may be adjusted in position to provide beams of continuously variable energy and intensity. By changing foil shapes as well, it has been possible to vary the ratio of intensities in the two extraction channels from 1:1 to 1:5000.

A full report of recent developments on the cyclotron and beam lines (Fig. 2) has been given by Dutto *et al.*<sup>6</sup>. In regular operation beam currents have been limited to 10  $\mu$ A up to now by the capacity of the temporary beam dump—although over 100  $\mu$ A was run for 45 minutes in a successful test in July 1977. 100  $\mu$ A beams should be regularly available by the end of the year when the permanent beam dump and thermal neutron facility (TNF) should be ready for operation. These will be capable of handling the 135 kW power in the 300  $\mu$ A beams which could be extracted at 450 MeV. The TNF, with a lead target in a D<sub>2</sub>O moderator, will be a potent source of thermal neutrons ( $\sim 5 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$ ) for scientists of various persuasions in the reactor desert of Western Canada.

Three secondary beams of pions and muons are currently produced at target T2 in the high intensity beamline BL1. The performance of these is summarised in Table 1. Three further lines are under construction: BL1B for low intensity protons, M11 for fast pions, and M13 for slow pions and muons (Fig. 2).

In addition to the regular ion source, a 'Lamb shift' polarised source has been operating since February 1976. It can provide 960

nA of H<sup>-</sup> ions, of which 120 nA can be accelerated and extracted with 75–80% reversible polarisation. The variable energy of this polarised beam makes it unique in the 200–500 MeV region; such is the demand for experiments that it has been scheduled for almost half the time since it became operational. For the past year a beam of almost monoenergetic polarised neutrons has also been available<sup>7</sup>. When the polarised proton beam hits a liquid deuterium target the neutrons emerging at 9° in the initial polarisation-beam plane are found to be between 40% and 75% polarised, depending on the initial proton energy (Fig. 3).

Developments planned for 1978 include extraction of proton beams in the 70–180 MeV range for isotope production and lower energy nuclear experiments, and the addition of a third harmonic component to 'flat-top' the RF wave. This will improve the beam quality and allow individual turns to be extracted with a consequent reduction in energy spread from the present 1 MeV FWHM to only 0.1 MeV, sufficient to distinguish many nuclear states.

### Basic research with pions and muons

An experiment of considerable topical interest this year has been a search on the M9 channel for muon decays by the process  $\mu^+ \rightarrow e^+ + \gamma$ . In 1964 the branching ratio for such decays, which lack the neutrinos appearing in the usual process  $\mu^+ \rightarrow e^+ + \nu_e + \bar{\nu}_\mu$ , was found to be  $< 2.2 \times 10^{-8}$ . This has been taken as evidence for the conservation of separate electronic and muonic lepton numbers. Recently, however, leptons heavier than the muon have been found<sup>8</sup>. If new leptons were to be coupled to both electrons and muons the conservation laws need not be exact

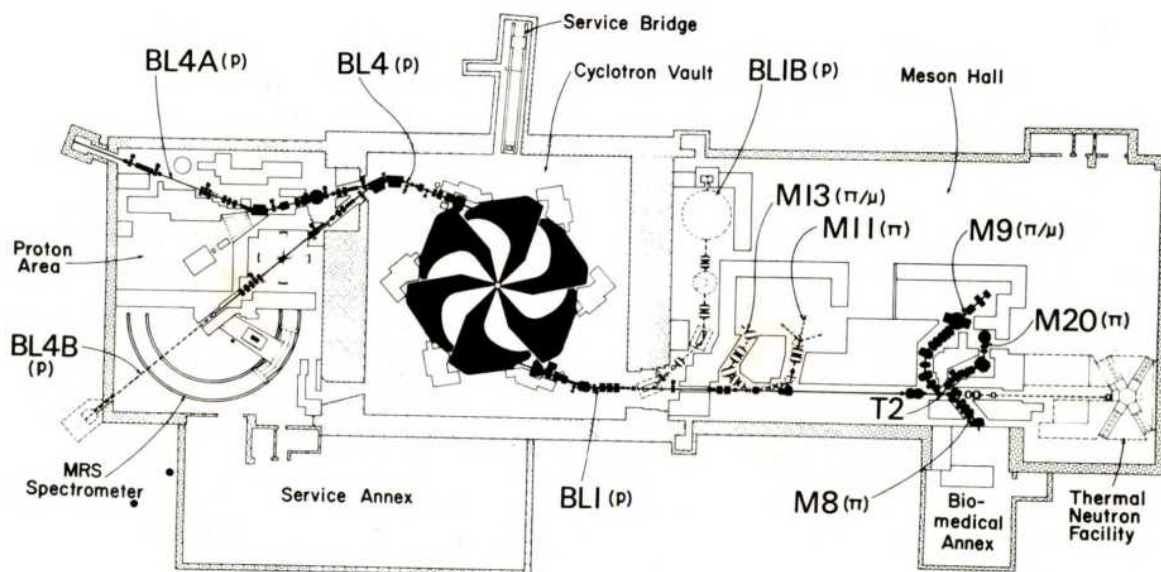


Fig. 2 Layout of the facility. Existing beam lines are indicated by solid lines, beam lines planned for future installation by dashed lines (see text).

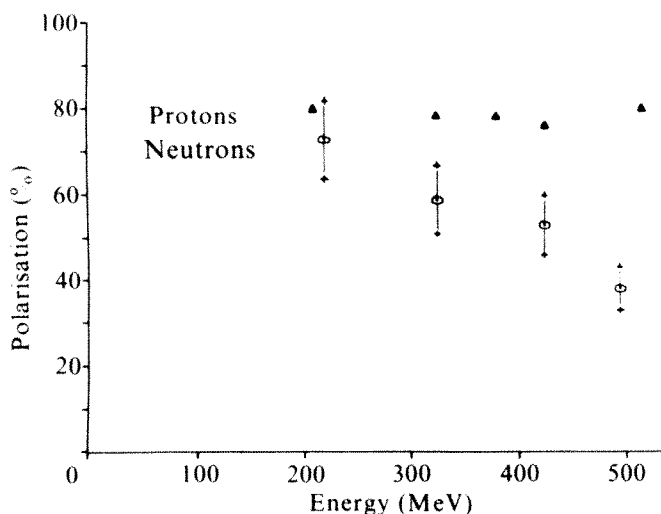


Fig. 3 Polarisation of the polarised proton (▲) and neutron (○) beams at various energies. The neutron values represent the spin rotation parameter  $R_s$  for the  $d(p,n)2p$  reaction multiplied by an assumed polarisation of 0.78.

and a finite fraction of muons could decay by the  $\mu \rightarrow e\gamma$  process (see, for example, ref. 9). An alternative mechanism<sup>10</sup> which could permit  $\mu \rightarrow e\gamma$  decays is exchange of Higgs bosons, particles postulated in modern gauge theories of the weak interaction but not yet observed experimentally. Theoretical estimates for these processes have put the branching ratio for  $\mu \rightarrow e\gamma$  in the range  $10^{-12} \rightarrow 10^{-8}$ .

To identify the decay process experimentally, two large sodium iodide crystals (46 cm and 36 cm diameters) were placed on opposite sides of the stopping target to measure the energies of electrons and  $\gamma$  rays. Analysis of the first runs has revealed one possible event and one background event, yielding an upper limit of  $3.6 \times 10^{-9}$  on the branching ratio at a 90% confidence level<sup>11</sup>.

Measurements are also being made of the branching ratios for the rare decays of the pion  $\pi \rightarrow e\nu$  and  $\pi \rightarrow e\nu\gamma$ . The former ratio provides a sensitive test of muon-electron universality, that is whether the muon and electron respond equally to the weak interaction.

Information on the threshold pion-nucleon ( $\pi$ -N) interaction has been obtained from studies of stopped pion charge exchange ( $\pi^-, \pi^+$ ) reactions in hydrogen, deuterium and  $^3\text{He}$ . At very low energies the 'strong' pion-nucleon ( $\pi$ -N) force is in fact relatively weak; its comparability to electromagnetic interaction strengths is demonstrated by the near-equality of the rates for the stopped pion charge exchange reaction  $\pi^-p \rightarrow \pi^0n$  in hydrogen and the radiative capture reaction  $\pi^-p \rightarrow \gamma n$ . The 'Panofsky ratio' of these two rates has now been remeasured at TRIUMF using the large sodium iodide crystals to identify the  $\pi^0$ s by their two decay  $\gamma$  rays. The value obtained<sup>12</sup> was  $1.546 \pm 0.009$ ; the precision of this result, twice that of previous work, helps to determine the  $\pi$ -N interaction at threshold more accurately.

In deuterium the charge exchange reaction  $\pi^-d \rightarrow \pi^0nn$  at rest is strongly suppressed by conservation laws. In fact the non-observation of this reaction provided early evidence that the  $\pi^0$  had the same (negative) parity as the  $\pi^-$ . The reaction is not totally prohibited, but for it to occur both the  $\pi^0$  and the two neutrons must be produced with non-zero orbital angular momentum. With only 1.09 MeV kinetic energy available such a final-state configuration is very unlikely. Using information from the  $\pi$ -N interaction at threshold the branching ratio has been estimated<sup>13</sup> to be  $1.49 \pm 0.10 \times 10^{-4}$ . The sensitivity of the present equipment has now enabled the reaction to be seen at rest for the first time<sup>13</sup> (Fig. 4) with a branching ratio of  $1.45 \pm 0.19 \times 10^{-4}$ , an order of magnitude smaller than the upper limit previously established, and in agreement with the theoretical estimate.

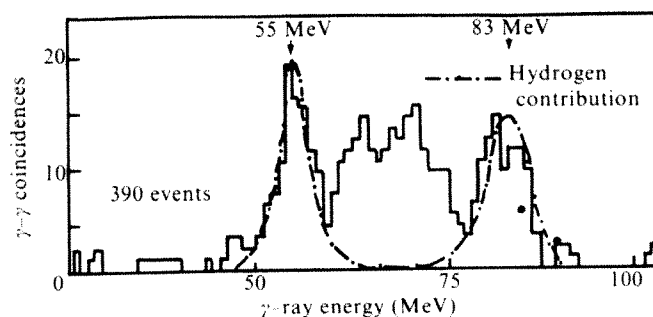
Studies of  $\pi$ -nuclear interactions are exemplified by an experiment on the elastic scattering of positive pions from  $^{12}\text{C}$ . This has

utilised the good quality low energy pion beams available on the M8 channel to study the relatively unexplored region below the (3,3) resonance, caused by excitation of individual nucleons to the  $\Delta(1232 \text{ MeV})$  state, and which dominates the scattering of pions between 70 and 400 MeV. Measurements were made<sup>14</sup> with 30, 40 and 50 MeV pions at scattering angles from  $15^\circ$  to  $150^\circ$ . This angular range includes the sensitive region of interference between Coulomb and nuclear scattering at these energies and should therefore provide a stringent testing ground for any theoretical models. In fact theoretical calculations<sup>14</sup> based on free  $\pi$ -N scattering are in poor agreement with the data unless nuclear absorption and Pauli exclusion effects are included; these effects have not been needed to account for data at higher energies. When 30 MeV negative pions were scattered from the same target the data clearly showed the difference between  $\pi^+$  and  $\pi^-$  Coulomb interference effects.

Muons which slow down and stop in matter will generally be captured by nearby atoms in the few microseconds before they decay. The events which take place in this short period make possible two fields of study, muonic X-rays and muon spin rotation ( $\mu\text{SR}$ ). Both are being actively pursued at TRIUMF, but but we shall concentrate on the latter. The muons in the beam are strongly spin-polarised as a result of conservation of angular momentum at their formation in pion decay ( $\pi \rightarrow \mu\nu$ ), the neutrino being inherently polarised while the pion has spin zero. The standard  $\mu\text{SR}$  technique consists in measuring the time interval between the stopping of a spin-polarised muon and the detection in some fixed direction of the electron emitted in its decay  $\mu \rightarrow e\nu\bar{\nu}$ . Since the electron tends to come off in the direction of the muon spin axis, a time spectrum accumulated from many  $\mu$  decay events will display oscillations with the frequency of precession of the muon spin in the local magnetic field.

$\mu\text{SR}$  studies involve three groups on channel M20. One group has concentrated on the behaviour of positive muons in ferromagnetic metals, where the muons are captured into interstitial positions in the lattice but can then diffuse from site to site. In cobalt (Fig. 5) temperature variations in the relative directions of internal dipolar and hyperfine fields lead to drastic changes in the  $\mu^+$  precession frequency and relaxation rate<sup>15</sup>. In a large extremely pure single crystal of iron ( $150 \times 48 \times 1.1 \text{ mm}$ ) precession was observed down to 23 K in zero applied field<sup>16</sup>; previously it had not been seen below 100 K. The results suggest high temperature diffusion of the  $\mu^+$  by thermally activated hops, superseded below 44 K by quantum tunnelling between sites. A second group uses 4.1 MeV 'surface' muons to produce muonium ( $\mu^+e^-$ ) atoms (Mu) in gaseous targets. When impurities such as  $\text{Cl}_2$ ,  $\text{HBr}$  and  $\text{O}_2$  are added the muonium precession signal shows a concentration-dependent quenching rate from which chemical reaction rate constants can be extracted, thus allowing a comparison of the chemistry of Mu, H and D atoms. The third group has found that Mu atoms formed when muons stop in fine silica powder diffuse rapidly out of the grains into the vacuum (J. B. Warren, personal communication). This paves the way for new studies using silica powders as a 'moderator gas' for chemistry experiments. In the realm of particle physics this discovery will be

Fig. 4 180° coincidence  $\gamma$ -ray spectrum obtained using two large NaI detectors, showing the characteristic signatures of the stopped pion charge-exchange reactions  $\pi^-p \rightarrow \pi^0n$  (wings) and  $\pi^-d \rightarrow \pi^0nn$  (centre—here observed for the first time).



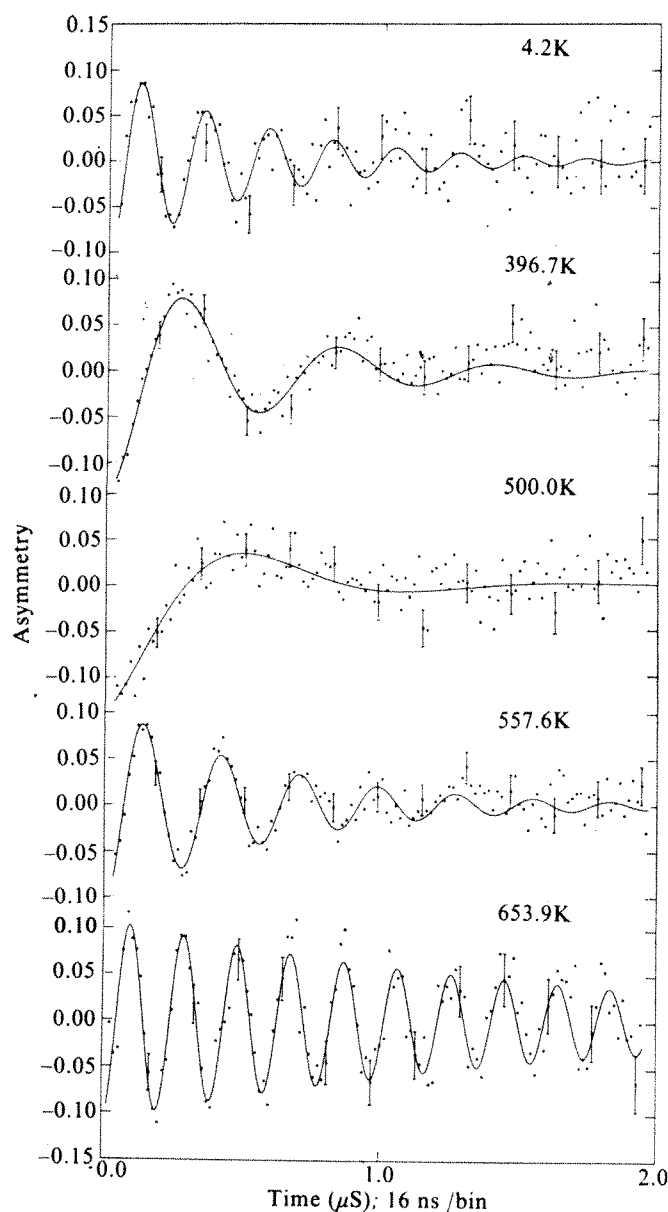


Fig. 5 Precession signals from positive muons in cobalt over a range of temperatures (with the muon decay folded out). The temperature variations in precession frequency and relaxation rate are directly related to variations in the relative directions of the internal dipolar and hyperfine magnetic fields in the cobalt.

applied to a search for muonium-antimuonium conversion in vacuum.

### Basic research with protons and neutrons

The availability of intense, variable energy, beams of protons and neutrons makes the study of nucleon-induced reactions an important aspect of the basic research programme at TRIUMF. The most fundamental of these reactions is of course the elastic scattering of one nucleon (N) by another, and this is the subject of a comprehensive series of measurements on beamline 4A. Because the force between two nucleons depends on the relative directions of their spins it is necessary to use either a spin-polarised beam or target if the spin dependence of the force is to be completely determined. In fact nine independent parameters are needed to describe N-N scattering above the inelastic threshold at 280 MeV, rather than the one needed for spin-zero particles.

Initial measurements have concentrated on the Wolfenstein parameters describing the changes in polarisation on scattering. For pp scattering polarised protons were directed into a liquid hydrogen target and their subsequent polarisation was measured using a large high-efficiency polarimeter. With the aid of a superconducting spin-precession solenoid the parameters  $P$ ,  $D$ ,  $R$  and  $R'$  were determined at a number of forward angles for five

energies between 209 and 515 MeV. These data have greatly improved the accuracy to which isospin  $T = 1$  N-N scattering is known at these energies<sup>17</sup>.

To study np scattering a polarised neutron beam was developed, as described above, to avoid the inaccuracies inherent in extracting pn from pd scattering data. In the process of this work the polarisation transfer parameters  $D_t$ ,  $R_t$  and  $R'_t$  for d(p,n)2p were measured<sup>7</sup>. (Compare Fig. 3). Measurements of the angular distribution of  $P$  and  $D_t$  in np scattering have been completed at a number of energies. The 325 MeV np data, together with the purely  $T = 1$  pp data, has enabled a long standing ambiguity in the  $T = 0$  N-N scattering to be removed<sup>18</sup>.

Pion production through the  $(p, \pi^+)$  reaction near threshold has been studied using both polarised and unpolarised proton beams on hydrogen, deuterium, beryllium and carbon targets. A 50 cm Browne-Buechner spectrograph was used to analyse pions with laboratory kinetic energies between 15 and 90 MeV. In the case of hydrogen ( $pp \rightarrow d\pi^+$ ) the measurements<sup>19</sup> for the first time give clear evidence of the importance of d- (as well as s- and p-) wave effects for pions with centre-of-mass energies from 60 MeV down to only 8 MeV.

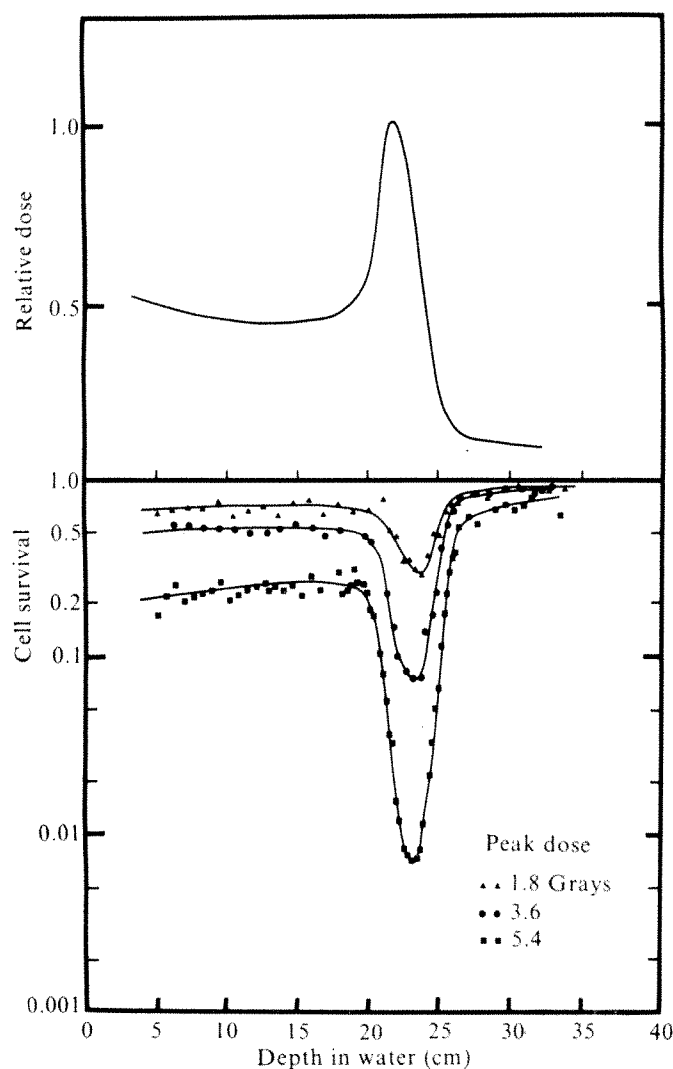
For 200 MeV protons on  $^9\text{Be}$  and  $^{12}\text{C}$  surprisingly high peak asymmetries ( $-65 \rightarrow -95\%$ ) were observed<sup>20</sup> for pions associated with both the ground state and groups of excited states of the residual nucleus; for instance, for the three states of  $^{13}\text{C}$  at 3.09, 3.68 and 3.85 MeV the unresolved pion asymmetry was  $-95\%$  at  $60^\circ$ . There is so far no theoretical explanation for these high negative values.

The scattering and reactions of protons on light nuclei are being studied on line 4B. At several hundred MeV the protons in the beam have much higher energies and speeds than those of the nucleons in a nucleus, which are therefore essentially stationary during an interaction. Furthermore the de Broglie wavelength of the incoming proton is smaller than the nuclear diameter so that it tends to interact with single or a few neighbouring nucleons rather than with the nucleus as a whole. The intermediate energy region is therefore a good one in which to test theoretical attempts to predict p-nucleus scattering from p-nucleon scattering, and to study pairing and clustering of nucleons. All in all the phenomena to be expected are rather different from those familiar at lower energies.

Experimentally,  $p\text{-}^4\text{He}$  scattering has been measured at both forward ( $4\text{--}14^\circ$ ) and backward ( $140\text{--}168^\circ$ ) angles. A surprise has been the high (85%) analysing power at  $160^\circ$  at 400 MeV. Another interesting polarisation effect was the observation<sup>21</sup> of J-dependence in  $(p, 2p)$  cross-sections for 200 MeV polarised protons incident on  $^{16}\text{O}$  (events involving proton knockout from the  $p_{1/2}$  shell could be separated from those from the  $p_{3/2}$  shell). In other experiments proton quasi-free and proton-deuteron quasi-elastic scattering from  $^{12}\text{C}$  have been studied. To improve the analysis of reaction products a medium resolution (0.5 MeV) spectrometer is being commissioned on this beam line. This matches the present  $\Delta E = 1$  MeV (FWHM) energy spread of the primary beam and is adequate to separate reactions proceeding via different excited states of many nuclei. A second stage is planned to improve this spectrometer resolution to 0.1 MeV to match the beam  $\Delta E$  of 0.1 MeV expected from separated turn operation of the cyclotron.

Nuclear chemistry studies in beam line 4 concern proton-induced light fragment emission and proton-induced fission. For the identification of the fragments a 5-detector 'fragment telescope' has been developed which is effective up to oxygen. Comparing the energy distribution of fragments from Ag for 200 to 500 MeV bombarding energies with those reported at 1 to 5 GeV it seems that the evaporation mechanism satisfactory at higher energies will not explain the 200 MeV data (R. G. Korteling, personal communication). Studies of proton-induced fission have used a gas jet radioactivity transport system. A time-of-flight spectrometer has also been commissioned. Proton-induced fission of uranium and spallation of iodine have provided neutron-rich and neutron-deficient Sb isotopes, whose  $\beta$ - and  $\gamma$ -ray emissions are being studied.





**Fig. 6** Survival of Chinese hamster cells (CH<sub>2</sub>B<sub>2</sub>)  $\pi^-$ -irradiated in 25% gel/medium. Top: The depth dose profile of the 90 MeV pion beam. Bottom: Survival profiles for cells irradiated in gel/medium at 0°C. Dose rate at peak approximately 2 rad min<sup>-1</sup>. Control plating efficiency 70%.

### Biomedical use of negative pions

Although the *raison d'être* of TRIUMF is research in basic science, an active programme is under way to explore possible applications of the various beams. At the moment the biggest of these projects is an investigation of the potentially favourable properties of negative pions for treating cancer, funded jointly by the British Columbia Cancer Foundation, Health and Welfare Canada, and the National Cancer Institute of Canada. Compared with neutral X-rays and  $\gamma$ -rays whose interactions fall off exponentially with depth and which therefore give their greatest dose near the skin, negative pions have a definite range in matter and give their greatest dose where they stop—mainly because of the local deposition of their rest mass energy (140 MeV) in 'pion stars' on absorption in a nucleus following capture into atomic orbits. Pions are therefore expected to be more effective for treating deep-seated tumours. Furthermore, the high linear energy transfer (LET) component of the radiation field produced by the pion stars decreases the oxygen enhancement ratio (OER) and thus reduces the inherent radioresistance of hypoxic tumour cells<sup>22</sup>. Clinical investigation of pion radiotherapy has had to await the coming into operation of intense pion beams (say  $10^8 \pi^- s^{-1}$  or more); for the M8 channel at TRIUMF this would require a primary beam of 100  $\mu A$  protons. The lower currents which have been available so far have, nevertheless, been useful

for preparatory studies. They fall into three general areas: measurement of the clinically important properties of the pion beam, dosimetry of the pion beam and biological measurements *in vitro* of the relative effectiveness of pion radiation<sup>23</sup>.

The stopping  $\pi^-$  flux in the M8 channel has been found to peak for 100 MeV pions; in water these have a range of 29 cm, within which 50% are lost by nuclear or electronic interactions. Sextupole magnets in the channel enable the spot size to be adjusted from  $1.2 \times 1.8 \text{ cm}^2$  to  $4 \times 15 \text{ cm}^2$  or  $10 \times 10 \text{ cm}^2$  while maintaining a uniform distribution. Contamination of the beam by muons is unimportant; electrons are dominant at low energies, but at 100 MeV the  $e^-/\pi^-$  ratio is only 0.18.

Dose fields in a water phantom can be automatically scanned in three dimensions under computer control through a CAMAC interface; the computer is also used to monitor the beam position and magnet settings, and to collect and display the data. A depth-dose profile for a 90 MeV pion beam is shown in Fig. 6. For a wide momentum bite the width of the peak can be increased to about 8 cm H<sub>2</sub>O. The best available beam tune provides about 20 rad h<sup>-1</sup>  $\mu A^{-1}$  protons over a volume of  $3 \times 3 \times 5 \text{ cm}$  in the stopping region. For measuring the RBE and OER factors in cell survival experiments, a gel suspension technique has been developed<sup>24</sup> which avoids the mixing problems of fluid media.

Measurements *in vitro* of cell survival, using this technique, were begun in 1976 when the proton current was raised to 5 and then 10  $\mu A$ . The results of irradiating Chinese hamster cells (CH<sub>2</sub>B<sub>2</sub>) with peak doses of 1.8, 3.6 and 5.4 Grays are shown in Fig. 6. From these preliminary measurements it seems that the biological properties of pion radiation differ little from those predicted. Measurements on biological systems *in vivo* have begun and clinical investigations will start approximately six months after the current is raised to 100  $\mu A$ .

### Applied research with protons

Three possible applications of proton beams are under study at present. Two of these—proton radiography and radioisotope production—are of medical interest; the third concerns the electromagnetic breeding of nuclear fuel.

The use of protons for radiography depends on their well defined range in matter. A detector placed near the end of the range of a monoenergetic proton beam will see large changes in intensity for only small changes in the thickness or density of the material traversed. For 200 MeV protons the enhancement factor can be as high as 50. A promising application for this 'marginal range radiography' is in the localisation of small tumours in soft human tissue, particularly as the dose would be relatively low. With a view to such applications preliminary experiments have been started at TRIUMF using a 200 MeV proton beam collimated down to 2 mm diameter (E. W. Blackmore, personal communication). The pencil beam is scanned in a raster fashion across the target using steering magnets under computer control. The protons transmitted may be detected on film or in a range telescope consisting of four thin plastic scintillators. Initial tests have been encouraging, showing, for example, that 0.25 mm steps in aluminium or perspex can readily be resolved. Work is now proceeding towards the development of a system capable of scanning over a  $10 \times 10 \text{ cm}^2$  area in 10 s for a total dose of less than 100 mrem.

Work on radioisotope production has concentrated on the development of a viable technique for producing <sup>123</sup>I (J. S. Vincent, personal communication). This is a highly desirable isotope for diagnostic nuclear medicine because its decay by electron capture enables it to deliver a specific dose only 1% of that from the conventional,  $\beta$ -emitting <sup>131</sup>I. 480 MeV protons are allowed to bombard a caesium heat pipe target producing xenon isotopes (among others) by spallation reactions; the caesium is heated above its melting point, freezing the xenon atoms which are then floated 18 m in a helium jet to a liquid nitrogen trap. After three <sup>123</sup>Xe half-lives the trap is warmed to remove long-lived Xe isotopes; a further delay clears <sup>121</sup>I, when the remaining iodine is distilled off from the daughter isotopes. The only significant contaminant is <sup>125</sup>I, present at the 0.3% level at

the end of bombardment. Unfortunately, this gives a specific dose only a little less than  $^{131}\text{I}$ , and it is much longer lived (59 d) than  $^{123}\text{I}$  (13 h). It is computed to double the dose from  $^{123}\text{I}$  (that is, from 1% to 2% of that from  $^{131}\text{I}$ ) in 28 h.

With the cooperation of Vancouver General Hospital 5 mCi of  $^{123}\text{I}$  produced in this way was used for a whole-body search for a metastatic thyroid tumour in a patient; the resolution was found to be superior to that from an  $^{131}\text{I}$  scan. It is now proposed to build a pilot plant for the regular production of 300 mCi  $^{123}\text{I}$  per week.

The third proton application under study is the electromagnetic breeding of nuclear fuel. Under contract to Atomic Energy of Canada Ltd, an experimental programme has begun to study the conversion of 'fertile'  $^{238}\text{U}$  and  $^{232}\text{Th}$  to fissile  $^{239}\text{Pu}$  and  $^{233}\text{U}$  respectively under proton bombardment. The high energy protons serve to produce an intense flux of spallation and evaporation neutrons; the conversion then proceeds through radiative neutron capture and two  $\beta$ -decays. The first step in this study (I. M. Thorson, personal communication) has been to measure the neutron flux produced when 350 and 450 MeV protons are stopped in small (2.5 to 4.0 cm diameter) cylinders of uranium and thorium. For this purpose the target is surrounded by a 1.8 m diameter water tank in which the thermal neutron flux distribution is measured. The total neutron capture rate in the water can then be estimated and the appropriate correction applied to

the integrated flux. The next step will be to determine the fertile-to-fissile conversion rates by measuring characteristic  $\gamma$ -rays emitted in the decay steps. These measurements will begin this autumn.

We thank all our colleagues at TRIUMF and the participating universities whose work we have had the privilege of reporting.

1. Michaelis, E. G. *IEEE Trans.* **NS-22**, 1385-1396 (1975).
2. Hagerman, D. C. *IEEE Trans.* **NS-24**, 1605-1610 (1977).
3. Vogt, E. W. & Richardson, J. R. *IEEE Trans.* **NS-13**, (4) 262-276 (1966).
4. Richardson, J. R. *Nucl. Instr. Meth.* **24**, 493-500 (1963).
5. Rickey, M. E. & Smythe, R. *Nucl. Instr. Meth.* **18**, 66-68 (1962).
6. Dutton, G. *et al.* *IEEE Trans.* **NS-24**, 1653-1655 (1977).
7. Amsler, C. *et al.* *Nucl. Instr. Meth.* **144**, 401-406 (1977).
8. Perl, M. L. *et al.* *Phys. Rev. Lett.* **35**, 1489-1492 (1975).
9. Cheng, T. P. & Li, L. F. *Phys. Rev. Lett.* **38**, 381-384 (1977).
10. Bjorken, J. D. & Weinberg, S. *Phys. Rev. Lett.* **38**, 622-625 (1977).
11. Depommier, P. *et al.* *Phys. Rev. Lett.* **39**, 1113-1116 (1977).
12. Spuller, J. *et al.* *Phys. Lett.* **67B**, 479-482 (1977).
13. MacDonald, R. *et al.* *Phys. Rev. Lett.* **38**, 746-749 (1977).
14. Johnson, R. R. *et al.* *Nucl. Phys.* (in the press).
15. Nishida, N., Nagamine, K., Hayano, R. S., Yamazaki, T., Fleming, D. G., Duncan, R. A. & Brewer, J. H. *Hyperfine Interactions* (in the press).
16. Nishida, N. *et al.* *Solid State Commun.* **22**, 235-239 (1977).
17. Axen, D. A. *et al.* *Lettiere di Nuova Cimento* **20**, 151-156 (1977).
18. Amsler, C. *et al.* *Phys. Lett.* **69B**, 419-421 (1977).
19. Jones, G. *Proc. Int. Conf. Nucleon-Nucleon Interaction* (American Institute of Physics, New York, in the press).
20. Auld, E. G. *et al.* *Bull. Am. Phys. Soc.* **22**, 590-591 (1977).
21. Kitching, P. *et al.* *Phys. Rev. Lett.* **37**, 1600-1602 (1976).
22. Raju, M. R., Ganapuran, M., Richman, C., Martins, B. J. & Barendson, G. W. *Br. J. Radiol.* **45**, 178-181 (1972).
23. Henkelman, R. M., Skarsgard, L. D., Lam, K. Y., Harrison, R. W. & Palcic, B. *Int. J. Rad. Oncol., Radiol. Phys.* **2**, 123-127 (1977).
24. Skarsgard, L. D. & Palcic, B. in *Proceedings of the 23rd International Congress of Radiology*, **2**, 447-454 (Excerpta Medica, Amsterdam, 1974).

## articles

### Primaeval melting of the Moon

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*There is evidence that the Moon melted completely 4,400 Myr ago, and between 4,000 Myr and 3,200 Myr ago had an internal magnetic field. But gravity could not have provided the heat of melting, and it must have come from short lived radioelements. Theory suggests the transuranics with atomic numbers between 114 and 126 may be relatively stable, and it is shown that these 'superheavy elements' fit the requirements of the early heat source in the moon.*

THERE is widespread evidence for primaeval melting and differentiation processes in the Solar System, for example, iron and some stony meteorites have melted. The Earth has an iron core which, from evidence of its past magnetic field, is at least 2,000-Myr-old, and has had a sialic (Si-Al) crust since 3,600 Myr ago. While geochemistry suggests that the core formation and differentiation occurred very quickly, in 100 Myr, after the earth's accretion, the arguments are inconclusive. Melting produced extensive lava flows, generated at depth on Mars, Mercury and Venus. Some crustal deformation and core

differentiation must have occurred, as small present-day magnetic fields are present. The asteroid Vesta also has a melted surface. But, except for certain meteorites, the dates of these meltings and the completeness of the differentiation are uncertain so it could be argued that the differentiations of the terrestrial planets could have been due to heating long after accretion had been completed. Nevertheless, the ubiquity of melting in the solar system is of great significance.

With large bodies like the Earth and Venus, it is possible that the final stages of accretion release gravitational energy which, if trapped as heat, is enough to melt the body provided that the heat is retained, and not radiated to space, by opaque dust clouds around the accreting objects<sup>1</sup>. Moreover, if melting did not take place during accretion, and the planet so formed was uniform in composition, the subsequent core formation would release comparable energy; in the case of Earth about twice as much as that released in its history from U, Th, and  $^{40}\text{K}$  radioactivity<sup>2</sup>. If the formation of the core were rapid, this energy would be adequate to heat Earth to its melting point and cause complete differentiation, although heat would be transported away by solid-state convection.

Other mechanisms of generating heat have been suggested; but as records of planetary melting and differentiation are fragmentary, evidence to decide between them does not exist. For example, Sonnett *et al.*<sup>3</sup> suggested that in an early T-Tauri phase of the Sun, intense solar wind magnetic fields could have heated the planets by induction. As their ferromagnetic silicates are semi-conductors, the induced currents would cause their electrical conductivity to increase rapidly; thus considerable magnetic energy could be deposited in a planet. Such electromagnetic heating seems particularly applicable to melting meteorites, for induction by poloidal magnetic fields is limited by the skin effect to small depth. But heating of stony meteorites would have driven out xenon; and as xenon<sup>4</sup> is still present there is a problem with this theory.

Therefore, although Earth has had, and other terrestrial planets may have had, for much of their histories molten cores and solid but slowly convecting mantles, their early thermal histories remain obscure. In contrast, we know a great deal about the first 1,000 Myr of the Moon's evolution<sup>5</sup>. The Apollo Moon landings have shown that a differentiation took place 4,400 Myr ago, producing the anorthositic highlands and the source region of the mare basalts<sup>6</sup>. They have shown that the moon's iron core was molten until at least 1,300 Myr had elapsed<sup>7</sup>, and that it must have an internal heat source sufficient to generate a lunar magnetic field. This dynamo field inferred from the palaeomagnetism of the crystalline rocks disappeared after 3,200 Myr, either because the core solidified or because the heat sources became inadequate to sustain dynamo action<sup>8</sup>.

We can, therefore, speculate on the primeval sources of heat, which melted the moon in its first few 100 Myr to form its differentiated crust and iron core. We will show that for an object the size of the Moon the gravitational energy released in accretion and from known radioactive elements is insufficient. There have been predictions that relatively stable superheavy elements exist: if so, they might have been an important heat source in the early solar system. If evidence for their existence were to be found, the chemistry of the Moon, planets and cosmos will have to be re-examined.

The giant pleochroic haloes found in terrestrial micas<sup>9</sup> have diameters of 150 to 200  $\mu\text{m}$ ; if due to the emission of  $\alpha$  particles this range implies energies up to 14 MeV, much more energetic than those from any known natural  $\alpha$ -emitting nuclei. Gentry's<sup>10</sup> explanation for this was that the rare earth inclusions within the haloes must have contained superheavy nuclei of which some fraction still exists. These monazite inclusions were bombarded by a proton beam and it was claimed<sup>11</sup> that among the X-ray lines excited, were those calculated for elements with  $Z = 114, 116, 124, 125, 126$  and  $127$ . Other experiments which have followed have all been negative<sup>12-14</sup>, although only two of them, those at Stanford and Harwell<sup>15</sup>, have used monazite crystals which were taken from giant haloes. It is argued that these later experiments fall short of the required sensitivity due to inhomogeneous distribution of superheavy elements<sup>16</sup>. An alternative explanation of the pleochroic haloes has been proposed<sup>17</sup>: radioactive nuclei of  $Z \sim 92$  emitting low energy  $\alpha$  particles which, striking water molecules trapped in the mica or in the inclusions produce knock-on protons of great enough ranges to account for the discoloured spheres. To support this hypothesis von Wimmersperg and Sellschop<sup>17</sup> assume that the inclusion contained a homogeneous mixture of monazite and water. We consider that mica could not contain enough water close enough to the inclusions to explain the spherical haloes. Furthermore, in his account of the phenomena of these giant haloes, Gentry<sup>10</sup> shows photographs of micas in which giant haloes and normal haloes due to U decay exist side by side about 100  $\mu\text{m}$  apart.

A second kind of evidence for the existence of superheavy nuclei is reported by G. N. Flerov<sup>18</sup>, who has studied spontaneous fission in meteorites and measured the number of neutrons emitted in each such fission. He found that the average number of neutrons per fission lies between 4 and 10, with 95%

confidence. This may be compared with  $< 3$  for the known spontaneously fissioning elements. Thus it seems that the number of neutrons per spontaneous fission for nuclei in meteorites is much greater than for any known nucleus. The number of neutrons per spontaneous fission increases with mass, suggesting that superheavy nuclei are responsible.

A third kind of evidence suggests that the existence of superheavy elements explains the anomalous abundances of isotopes of xenon in carbonaceous chondrites which have a pattern inconsistent with yields from fission of U, Pu, Cm, and Cf<sup>19,20</sup>.

## Molten Moon and lunar magnetic field

We now develop our view that the moon is crucial for understanding early melting processes because its primeval history has been preserved. Urey<sup>1</sup> presented convincing arguments that the Moon was formed by accretion, recognising that it was probably too small to have been melted by gravitational energy, and argued that it had remained undifferentiated and was therefore a key to the origin of the solar system. This insight is still fundamentally valid, even though fragments of anorthosite found in the Apollo sampling of the regolith, and identified as originating in the highlands showed that the moon had differentiated early to form a highland shell of anorthosite gabbroic composition<sup>21</sup>. Geochemists<sup>22</sup> then calculated that only the top few hundred kilometres of the primitive Moon had to be melted to provide the observed thickness of this shell, and unlike the earth this differentiation could be securely dated; it occurred 150 Myr after the origin of the Moon. So, in the absence of conclusive evidence of the cause of melting, it seems reasonable to suggest, despite the uncertainties, that the cause was the last gravitational energy released when the Moon began to assume its present size<sup>6</sup>.

It has been widely assumed that the velocity of the incoming material would equal the escape velocity. For the Moon today, this is  $2.3 \text{ km s}^{-1}$ , so that the  $2.8 \times 10^3 \text{ Jg}^{-1}$  of material accreted would have been the energy released in the final stages of the moon's formation. As the energy required for melting silicates from 0 K is only  $2 \times 10^3 \text{ Jg}^{-1}$ , it has been accepted that this energy, if none were lost, could have been effective in melting the outer part of the Moon.

There are also strong arguments suggesting that the Moon melted to the centre a few hundred Myr after its origin. The mare basalts have crystallisation ages between 3,200 and 3,900 Myr, but their Pb-Pb model ages are about 4,400 Myr (ref. 22). Rb-Sr systematics<sup>23</sup> and samarium-neodymium decay<sup>24</sup> give similar model ages which are interpreted in terms of a closed-system melting<sup>25</sup>. The original differentiation, providing the source material of the lunar basalts, occurred about  $4.4 \times 10^3$  Myr ago and later this melted again to provide the magma which filled the maria between  $3.9 \times 10^3$  and  $3.2 \times 10^3$  Myr ago. The closed-system melting leads to a geophysical model which has three shells: an outer anorthosite shell over a basalt-rich shell, below which is the olivine or pyroxinite mantle<sup>26,27</sup> surrounding the core. The variations of electrical conductivity with depth, the seismic data<sup>28</sup>, and the moment of inertia<sup>29</sup>

Table 1 Isotope heating and melting of the Moon

Assumptions:

1.  $C_p = 0.20 \text{ cal g}^{-1} \text{ } ^\circ\text{C}$
  2.  $\Delta H_{\text{fusion}} = 100 \text{ cal g}^{-1}$
  3. Potassium abundance = 0.1%
  4.  $^{40}\text{K}$  abundance 4,600 Myr ago = 0.151%
  5.  $^{40}\text{K}$  half-life =  $1.25 \times 10^9 \text{ yr}$
  6. Heat of  $^{40}\text{K}$  decay = 0.8 MeV
  7. Melting point of the Moon =  $1,500 \text{ } ^\circ\text{C}$
  8. Total heat to melt the Moon =  $455 \text{ cal g}^{-1}$  ( $1.9 \times 10^{10} \text{ erg g}^{-1}$ )
- Initial heat rate =  $3.94 \times 10^{-7} \text{ cal g}^{-1} \text{ yr}^{-1}$   
Compute time to melt,  $t$ :

$$455 = 3194 \times 10^{-7} \int_0^t \exp(-0.693 t / 1.25 \cdot 10^9) dt$$

where  $t = 1,800 \text{ Myr}$



Table 2 Other radio-isotopes, half lives, and effectiveness

Radio isotope	Estimated Initial Abundance (No. of atoms per g of MgSiO <sub>3</sub> )	Half life (Myr)	Absorbable* disintegration energy (MeV)	Effectiveness† relative to <sup>40</sup> K
<sup>129</sup> I	$3 \times 10^{14}$	17	0.075	0.09
<sup>244</sup> Pu	$0.2 \times 10^{14}$	76	15	0.26
<sup>26</sup> Al	$1.5 \times 10^{21}$	0.74	1.6	$2.2 \times 10^8$
<sup>60</sup> Fe	$6 \times 10^{19}$	0.3	3	$4.0 \times 10^7$
<sup>238</sup> U	$2 \times 10^{14}$	$4.5 \times 10^9$	48.3	0.96
<sup>235</sup> U	$2 \times 10^{14}$	$0.72 \times 10^9$	43.3	
<sup>40</sup> K	$2.3 \times 10^{16}$	$1.25 \times 10^9$	0.8	1

Thus after 10 Myr, <sup>60</sup>Fe is negligible and <sup>26</sup>Al is down to the <sup>40</sup>K level.

\* Neutrinos escape.

† Initial heating rate relative to <sup>40</sup>K.

are all indicative of, or consistent with, the existence of a basalt-rich shell, the source of the mare lavas. Thus the moon must have melted to a much greater depth to have produced this differentiation than it would have if the differentiated shell were only anorthosite rock (Ca Al<sub>2</sub> SiO<sub>8</sub> plus other low-melting substances) which is only 60 km thick on the near side of the Moon.

The strongest evidence for a completely molten early moon is provided by the lunar gravitational and magnetic data. It used to be generally accepted that there was no iron core in the moon; its mean density, 3.34, is that of olivine. The existence of an iron core was first suggested in a theory of the non-hydrostatic shape of the moon, proposing that it is dynamically maintained by its finite strength<sup>30</sup>. The two-cell pattern of convection necessary to explain the shape seems compatible only with the existence of a core radius between 0.1 and 0.3% of the lunar radius<sup>30</sup>. More recently, evidence for the existence of an iron core has come from the extensive remanent magnetism found in the lunar surface rocks. Most of the samples of basalt and high-grade breccia possess a stable, natural remanent magnetism, and the magnetic anomalies mapped<sup>31</sup> by orbiting satellites are consistent with the laboratory findings. The directions of the magnetisation of the lunar crust must be such that no dipole field outside the moon exists, for an exceedingly small limit to this has recently been set by the Apollo 15 and 16 subsatellite magnetometer measurements<sup>32</sup>. This fact can be interpreted on the theory that the lunar shell was magnetised by an internal field<sup>33</sup>. Although other explanations for an internal field have been suggested, the dynamo mechanism in which fluid motions in a liquid iron core are responsible seems to be the only tenable one. Three lines of evidence, none as yet conclusive, are compatible with an iron core of 400–500 km radius existing in the moon. This data suggesting a conducting or dense core is obtained from electromagnetic induction<sup>34,35</sup>, from seismology<sup>28</sup>, and from the moment of inertia factor<sup>29</sup>, respectively. Tozer<sup>36</sup> has rediscussed the accretion process and concludes that gravitational energy can be released in the deep interior by shock waves from impacts of accreting material. The argument concerning this energy source has assumed that the accreting material falls to the growing moon from infinite distance. Instead, Ransford<sup>37</sup> has set up realistic models in which the moon accretes from material orbiting around the Sun at about 1 AU and from material thrown into it from other parts of the solar nebula by perturbations. He concludes, using experimental data on the effectiveness of impacts of convection of less kinetic energy into heat during impacts, that only a fraction of the outer 50 km of the moon would have melted through gravitational energy.

The magnetic maps of the far side of the moon show large anomalies over the deep basins; thus it seems likely that parts of the anorthosite highland shell have magnetisations greater by an order of magnitude than those of the mare basaltic flows<sup>38</sup>. This suggests that the lunar magnetic field was present a few hundred Myr after the lunar origin and existed until at least 3,200 Myr ago, after which, at an unknown time, the

dynamo action ceased. Recent studies<sup>39</sup> of the palaeointensity of this field suggest that it was about 1 G at 4,000 Myr ago and had diminished to about 0.03 G at 3,200 Myr ago.

Thermodynamic arguments on the heat required to maintain a dynamo generating such a strong field from such a small core show that more powerful heat sources were present than those which could have been due to U, Th, and <sup>40</sup>K, assuming their concentrations to be the same as in the chondritic meteorites. The energy to maintain a molten core and drive the palaeodynamo had to be supplied quickly or else solid state creep in the silicate mantle rather than conduction would have been the dominant thermal transport process and would have prevented the internal temperature from reaching the melting point<sup>40</sup>. The convecting core is a heat engine in which the 'useful' work done is the Joule heat dissipated by the electric currents in it. The efficiency of this dynamo cannot be greater than the fractional difference between the temperature at the centre and outside of the core multiplied by a factor equal to 2/5 for a uniformly distributed heat source<sup>41</sup>. From the adiabatic gradient in the core, this efficiency can be calculated, it is 0.016. A minimum value for the heat dissipated by toroidal currents to give the palaeofield can be estimated, it is  $4.8 \times 10^9$  W at 4,000 Myr ago (near that in the Earth today<sup>42</sup>). Assuming, as a minimum, that the Joule heat dissipated in generating the toroidal field is twice that given above, the minimum heat generated in the core 4,000 Myr ago was  $10^{10}$  W<sup>43</sup>.

### Radio-elements and speculative transuranics

Table 1 gives the time of melting the Moon from <sup>40</sup>K, assuming no heat loss and 0.1% abundance. Other radio-isotopes, their

Fig. 1 Barrier penetration Factor B against  $\alpha$ -particle energy in MeV.

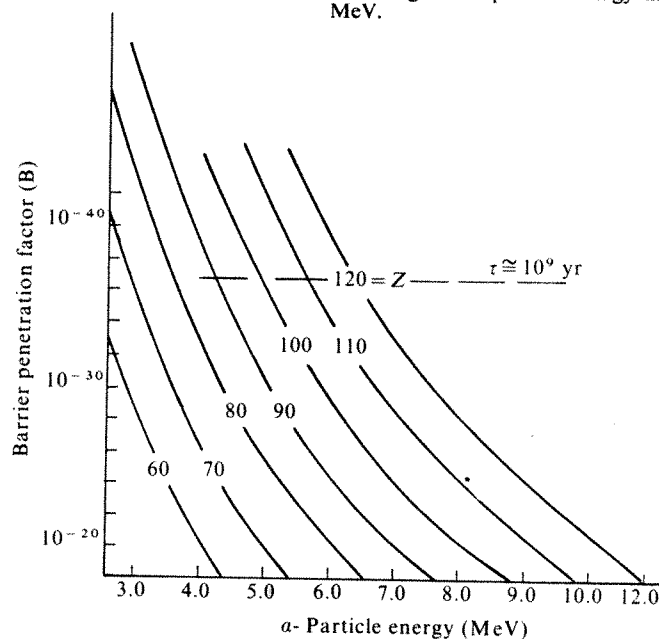


Table 3 Quantum numbers of the heavier elements

$n + 1$	$n$	1	Element or Z	Likely chemistry*
8	5	3	Ac, Th, Pa, U, Np, Pu, Am, Cm, Bk, Cf, Ei, Fm, Md. 102	{ Actinides, similar to the lanthanum group
	6	2	(103), (104), (105), (106), (107), (108), (109), (110), (111), (112)	{ Similar to hafnium through mercury
	7	1	(113), (114), (115), (116), (117), (118)	{ Similar to thallium through radon element 114 should be similar to lead
	8	0	(119), (120)	119 should be an alkali metal and 120 an alkaline earth
9	5	4	(121), (122), (123), (124), (125), (126), (127), (128), (129), (130), (131), (132), (133), (134), (135), (136), (137), (138)	Similar to actinium and lanthanum

\* Elements which should be soluble in iron are  $Z = 108, 109, 110$ , and possibly  $Z = 114, 115, 116$ .

half lives, and their effectiveness relative to  $^{40}\text{K}$  are listed in Table 2. We conclude that radio-element heating in the Moon by  $^{40}\text{K}$ ,  $^{26}\text{Al}$ , and  $^{235,238}\text{U}$  was sufficient to melt the Moon and thus produce a molten core, but on a time scale an order of magnitude too long, also none of these elements are soluble in iron and so cannot power a dynamo. The heat contribution from the short-lived (0.3 Myr)  $^{60}\text{Fe}$  (ref. 44) was 50% complete in the first 0.3 Myr and was sufficient to melt the core if the Moon had formed early enough<sup>45</sup>, and the same applies to  $^{26}\text{Al}$ , evidence for the existence of which in the early solar system has recently been discovered<sup>46</sup>. But most dynamic calculations<sup>47</sup> show that the time of formation of lunar sized objects by accretion from the solar nebula is about 100 Myr and it is probable that a similar time elapsed between the end of nucleogenesis and the formation of the nebula by contraction from an interstellar dust cloud<sup>48</sup>.  $^{244}\text{Pu}$  might be the source if its abundance were substantially higher than the estimate made here<sup>49</sup>, but it too is not soluble in iron.

Next we turn our attention to potential heating by the transuranics. Supposing elements of  $114 \leq Z \leq 127$  exist, what would be their chemical properties? These are indicated in Table 3, bearing in mind that no one has yet examined experimentally the chemistry of the elements filling the g valence electrons. The assignment of the transuranics to an order of filling of the s, p, d, f, and g electron shells (Table 3) allows us to predict that the elements which should be soluble in the iron core of the planets and moons have  $Z = 108, 109, 110$ , and

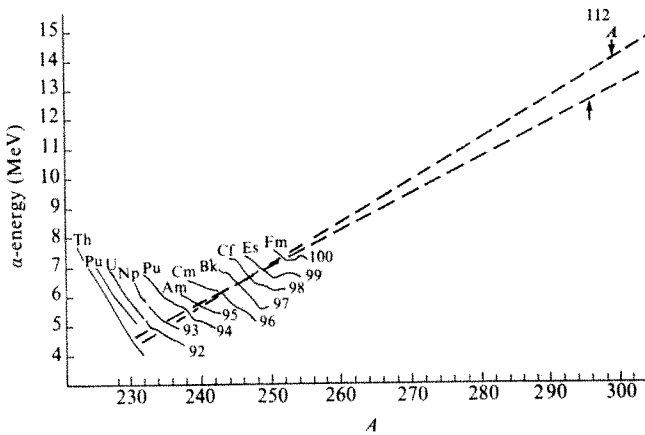
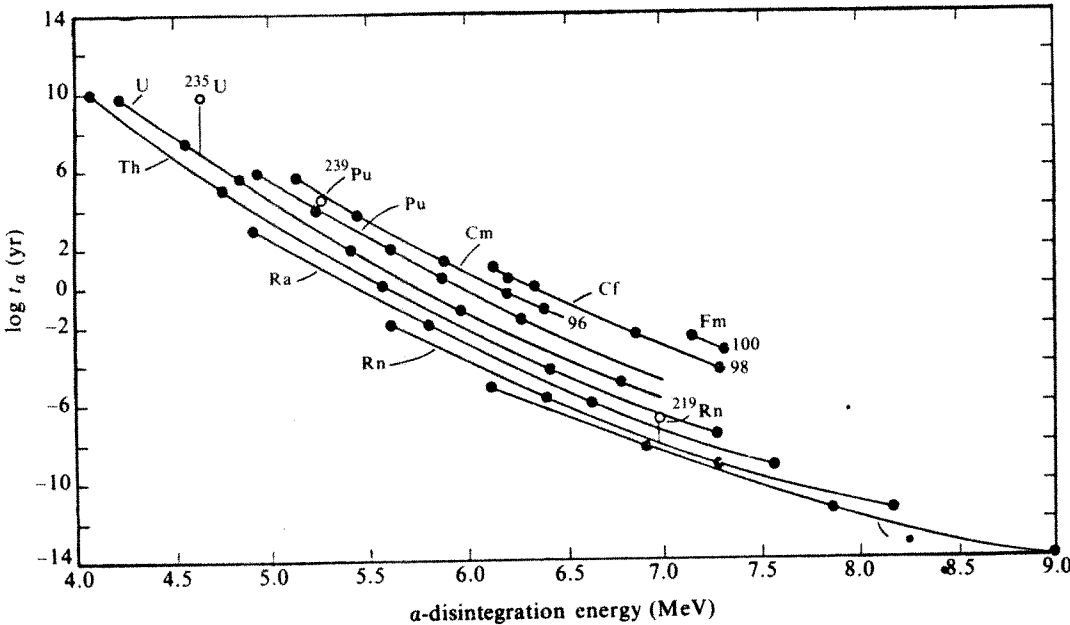


Fig. 2 Energy of  $\alpha$  emission against atomic number  $A$ .

possibly 114, 115, and 116. If any of these have lifetimes sufficiently long, then we may have found a heat source soluble in iron which might, therefore, be important for producing an early magnetic field.

What can be said about the stability of elements  $114 \leq Z \leq 128$  against  $\alpha$ -particle emission? Straight forward application of the Gamow-Gurney-Condon theory of  $\alpha$  emission by quantum mechanical tunnelling yields the systematics for the barrier

Fig. 3 Relation between half life for  $\alpha$  emission and  $\alpha$ -particle energy.



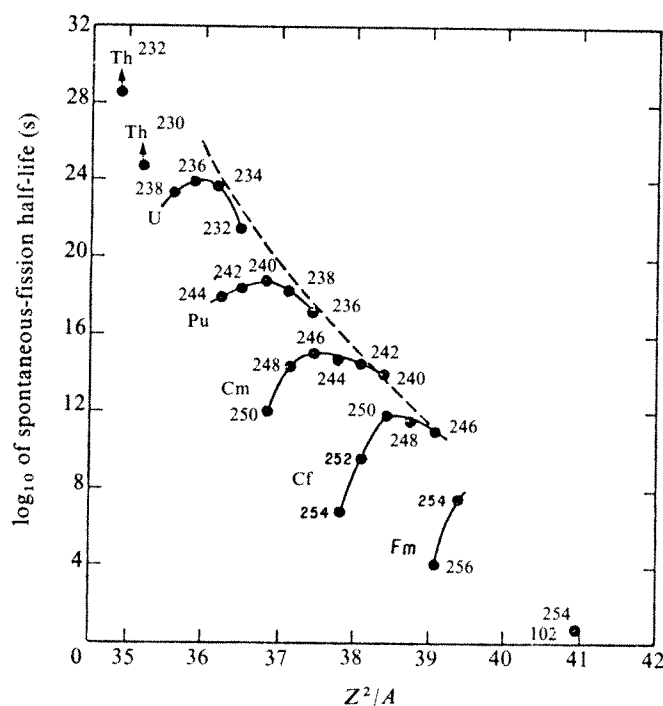
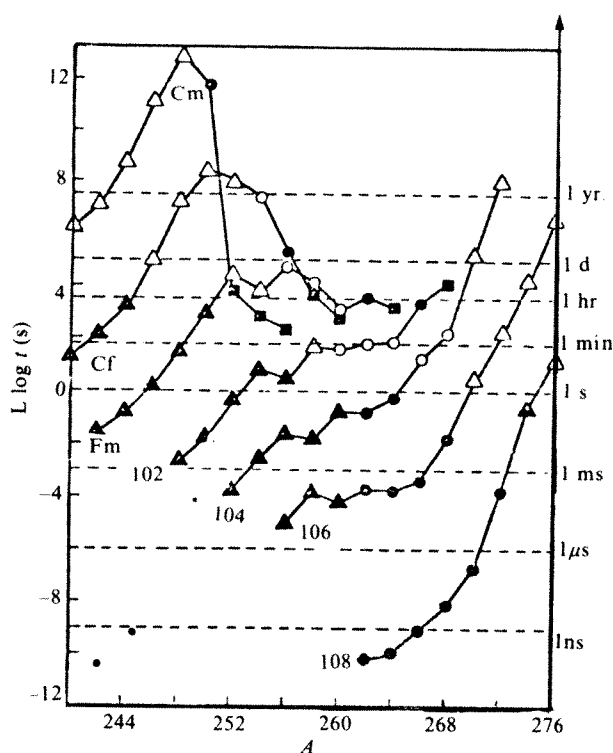


Fig. 4 Relation between half life for spontaneous fission and  $Z^2/A$ .

penetration factor  $B$  shown in Fig. 1 (adapted from ref. 50). The lifetime of  $Z \sim 114$ , for example, must be at least 1,000 Myr. Assuming the  $\alpha$ -particle velocity to be  $\sim 10^9 \text{ cm s}^{-1}$  inside a nucleus of diameter  $\sim 10^{-12} \text{ cm}$ , the number of collisions against the Coulomb barrier is  $\sim 10^{21} \text{ s}^{-1}$ , so to obtain a lifetime of  $\sim 1,000 \text{ Myr}$  requires a barrier factor of,  $B = (10^9 \times 3 \times 10^7) (10^{21}) = 3 \times 10^{37}$ .

According to Fig. 1, for  $B = 3 \times 10^{37}$  and  $Z \sim 114$ , the  $\alpha$ -particle energy must not exceed about 5.7 MeV. Then the giant haloes, of diameters indicating  $\alpha$  energies up to 14 MeV, must have been caused by  $\alpha$  decay of the daughters of the trans-

Fig. 5 Relation between total half life and  $A$ .



uranics still existing, but not by the elements of  $10^2$ – $10^3$  Myr lifetimes themselves.

Let us consider the  $\alpha$ -particle systematics of the known transuranics. The known  $\alpha$ -particle energies are plotted against  $A$  in Fig. 2 (adapted from ref. 51). For elements  $90 \sim Z \sim 100$ , the trend is for increasing  $\alpha$  energy with increasing  $Z$ , with an indicated  $\alpha$  energy of 11–12 MeV for element  $^{114}284$ . Therefore, the trend is for even shorter lifetimes with increasing  $Z$  as shown in Fig. 3 (ref. 51). The same is true of decay by spontaneous fission, see Fig. 4 (refs 51 and 52). How could the theoreticians<sup>53–56</sup> reverse these trends and so account for lifetimes of  $\sim 10^2$ – $10^3$  Myr by  $\alpha$  decay? They must keep the  $\alpha$  energy below 5.7 MeV. The  $\alpha$  energy is given by (for example, element  $^{114}284$ ) the following expression for conservation of energy.

$$E = M(^{114}284) - M(^{112}280) - M(\alpha)$$

If a theoretical method can be devised to decrease the mass of the parent and increase the mass of the daughter nucleus, then  $E$  can be decreased below the required limit of 5.7 MeV. In practice, energies of nuclear cores are computed for shapes varying from spherical through a sequence of prolate and hour-glass deformations. For each shape there is a corresponding nuclear potential from which single particle wave functions and their energies are evaluated using the Schrödinger equation. The sum of the core energy and the energies for the single particle wave functions give  $M(^2A)$ . By using nuclear potentials giving small values of  $M(^2A) - M(^2A-4)$ , the theoreticians<sup>53–56</sup> have been able to keep  $\alpha$  energies below 5.7 MeV and thereby account for lifetimes as long as 1,000 Myr against  $\alpha$  decay. In the same *ad hoc* way, prolate and hour-glass potentials for the ground state account for sufficiently long lifetimes against decay by spontaneous fission<sup>52–56</sup>. The trends of lifetime obtained by this kind of postulation are shown against neutron number and mass (see ref. 22).

A recent calculation of lifetimes of superheavy elements has been made by Möller and Nix<sup>56</sup> adding to energies computed from a liquid drop model, fine corrections from shell model calculations of energies of single particle states in various potential energy functions. They find for  $Z = 126$ ,  $N = 228$  a lifetime too short (20 yr) for it to be found in nature. To calculate a lifetime 1,000 Myr, major changes have to be made in the nuclear model, for example, making the nucleus toroidal. For elements of  $Z \sim 115$  small changes in the theory allow lifetimes to be sufficiently long. (A review of the state of the theory has been given by Hodgson<sup>57</sup>).

In short, the Moon could be melted by  $^{40}\text{K}$  and  $\text{U}$  in the first 2,000 Myr, but iron soluble heat sources seem to require the long-lived transuranics.  $^{60}\text{Fe}$  could melt the Moon only if it assembled very early in less than 20 Myr and could not generate the magnetic field. We estimate that about  $10^{-5}$ – $10^{-7} \text{ J g}^{-1} \text{ yr}^{-1}$  are required to keep the dynamo going. This would extend the effectiveness of  $^{60}\text{Fe}$  to about 8.3 Myr. Ray and Kohman<sup>14</sup> estimates a factor of uncertainty of 3 in the  $^{60}\text{Fe}$  half life. So this number might be as long as 25 Myr, far short of the required time.

## Conclusion

There is an acute problem in obtaining, using conventional ideas, strong enough heat sources to melt the Moon 4,400 Myr ago and to generate its magnetic field in an iron core. We conclude that long-lived radioactive isotopes were present in the early Moon, although of their existence there is as yet little evidence. We consider that the elements of the 'island of stability' could have melted the early Moon and, as they may be soluble in iron, in driving the core dynamo. If so they must have half lives of 100–1,000 Myr. If they exist, many aspects of the origin of the solar system will require rethinking.

That these fundamental questions have emerged in the study of the Moon shows the key importance of the primaeval record that it has retained in its crust, which is much more complete than in any other planet, and also of the returned samples from



the Apollo and Luna missions have made possible radioactive age determinations and palaeomagnetic studies. This work reinforces the case for further fundamental studies of the Moon<sup>5b</sup>.

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1. Urey, H. C. *The Planets. Their Origin and Development* (Yale University Press, New Haven, 1952).
2. Runcorn, S. K. *Phil. Trans.* **258**, 228 (1965).
3. Sonnett, C. P., Colbourne, D. S. & Schwartz, K. *Icarus* **24**, 231–255 (1975).
4. Reynolds, J. H. *J. geophys. Res.* **68**, 2939–2956 (1963).
5. Brown, G. M., Eglinton, G., Runcorn, S. K. & Urey, H. C. (eds), *The Moon: A New Appraisal from Space Experiments and Laboratory Analyses* (The Royal Society, London, 1977).
6. Taylor, S. R. *Lunar Science: A Post-Apollo View* (Pergamon, Oxford, 1975).
7. Runcorn, S. K. *et al. Proc. R. Soc. A* **325**, 157–174 (1971).
8. Collinson, D. W., Stephenson, A. & Runcorn, S. K. *Phil. Trans. R. Soc. A* **285**, 241–247 (1977).
9. Rutherford, E., Chadwick, J. & Ellis, C. D. *Radiations from radioactive substances* (Cambridge University Press, Cambridge, 1950).
10. Gentry, R. V. *Science* **169**, 670–673 (1970).
11. Gentry, R. V. *et al. Phys. Rev. Lett.* **37**, 11–14 (1976).
12. Stephan, C., Epherry, M., Cieslak, E. & Sowinsky, M. *Phys. Rev. Lett.* **37**, 1534–1536 (1976).
13. Jelley, N. *et al. Nature* **265**, 35–36 (1977).
14. Kettle, B. H., O'Kelley, G. D., Stoughton, R. W. & Halperin, J. *Phys. Rev. Lett.* **37**, 1734–1737 (1976).
15. Sparks, C. J., Raman, S., Yakel, H. L., Gentry, R. V. & Krause, M. O. *Phys. Rev. Lett.* **38**, 205–208 (1977).
16. Cahill, T. A. *et al. Phys. Rev.* (in the press).
17. von Wimmersperg, U. & Selischop, J. P. F. *Phys. Rev. Lett.* **38**, 886–888 (1977).
18. Hodgson, P. *New Scientist* **74**, 706 (1977).
19. Larimer, J. W. & Anders, E. *Science* **175**, 981–982 (1972).
20. Flynn, G. J. & Loubet, M. *Nature* **268**, 717–718 (1977).
21. Wood, J. A., Dickey, J. S. Jr., Marvin, V. B. & Powell, B. N. *Geochim. cosmochim. Acta Suppl.* **1**, 965–988 (1970).
22. Hubbard, N. J. & Minear, J. W. *Geochim. cosmochim. Acta Suppl.* **6**, 1057–1085 (1975).
23. Papanastassiou, D. A. & Wasserburg, G. J. *Geochim. cosmochim. Acta Suppl.* **6**, 1467–1490 (1975).
24. Lugmair, G. W., Scheinin, N. B. & Marti, K. *Geochim. cosmochim. Acta Suppl.* **1**, 1419–1429 (1975).
25. Urey, H. C., Marti, K., Hawkins, J. W. & Lir, M. R. *Geochim. cosmochim. Acta Suppl.* **1**, 965–988 (1971).
26. Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **7**, 3221–3228 (1976).
27. Sonnett, C. P. & Duba, A. *Nature* **258**, 118–121 (1975).
28. Nakamura, Y. *et al. Geophys. Res. Lett.* **1**, 137–140 (1974).
29. Blackshear, W. T. & Capcynski, J. P. *J. geophys. Res.* **82**, 1699–1701 (1977).
30. Runcorn, S. K. *Proc. R. Soc. A* **296**, 270–284 (1967).
31. Coleman, P. J. & Russell, C. T. *Phil. Trans. A* **285**, 489–506 (1977).
32. Russell, C. T., Coleman, P. J. & Schubert, G. *Science* **186**, 825–826 (1974).
33. Runcorn, S. K. *Phys. Earth Planet. Int.* **10**, 327–335 (1975).
34. Goldstein, B., Phillips, R. S. & Russell, C. T. *Geophys. Res. Lett.* **3**, 289–292 (1976).
35. Sonnett, C. P. & Wiskerchen, M. J. *Geochim. cosmochim. Acta Suppl.* **8**, (1977).
36. Tozer, D. C. *The Origin of the Solar System* (ed. Dermott, S.) (in the press).
37. Ransford, C. A. *Lunar Science Abstracts VIII* 793–794 (1977).
38. Lin, R., Anderson, K. A., Bush, R., McGuire, R. E. & McCoy, J. E. *Geochim. cosmochim. Acta Suppl.* **7**, 2691–2703 (1976).
39. Stephenson, A., Collinson, D. W. & Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **5**, 2859–2871 (1974).
40. Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **6**, 2943–2953 (1975).
41. Backus, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1555–1558 (1975).
42. Gubbins, D. *Geophys. J.* **47**, 19–39 (1976).
43. Runcorn, S. K. *Science* (in the press).
44. Ray, J. C. & Kohman, T. P. *Canad. J. Phys.* **35**, 649–655 (1957).
45. Mizutani, H., Matsui, J. & Takeuchi, H. *The Moon* **4**, 476–489 (1972).
46. Lee, T., Papanastassiou, D. A. & Wasserburg, G. J. *Geophys. Res. Lett.* **3**, 109–112 (1976).
47. Kaula, W. M. & Harris, A. *Icarus* **24**, 516–524 (1975).
48. Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **8**, 463–469 (1977).
49. Lugmair, G. W. & Marti, K. *Earth planet. Sci. Lett.* **35**, 273–284 (1977).
50. Enge, H. A. *Introduction to Nuclear Physics* 290 (Addison-Wesley, Reading, Mass. 1966).
51. Friedlander, G., Kennedy, J. W. & Miller, J. M. *Nuclear and Radiochemistry* 2nd ed. 229 (Wiley, New York, 1955).
52. Viola, V. E. Jr & Seaborg, G. T. *J. inorg. Chem.* **28**, 741–761 (1966).
53. Nix, J. R. *et al. Rev. Nucl. Sci.* **22**, 65–120 (1972).
54. Fiset, E. O. & Nix, J. R. *Nucl. Phys. A* **193**, 647–671 (1972).
55. Möller, P. & Nix, J. R. *Phys. Rev. Lett.* **37**, 1461–1464 (1976).
56. Seaborg, G. T. & Bloom, J. L. *Sci. Am.* **220**, 57–67 (1969).
57. Hodgson, P. *New Scientist* **75**, 152–153 (1977).
58. Runcorn, S. K. & Coleman, P. J. *Nature* **265**, 197–199 (1977).

# Granulite xenoliths from Lesotho kimberlites and the lower continental crust

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*Six clinopyroxene–almandine granulite facies xenoliths have been analysed for major, trace and rare earth elements. Preliminary microprobe analyses suggest an origin near the base of the crust at temperatures of 660–830 °C and pressures > 9 kbar. Major element analyses indicate minimal chemical variation from a basaltic composition while the trace and rare earth element variation may be attributed to either magmatic or metasomatic activity.*

INFORMATION about the composition of the upper mantle has been obtained from studies of ultra-basic xenoliths brought up by kimberlites. In contrast, relatively little work has been carried out on the crustal xenoliths, particularly in defining deep seated varieties. The crustal xenolith suite in Lesotho kimberlites includes sediments and regional metamorphic rocks, comparable with those exposed in basement complexes, as well as basic garnet clinopyroxene granulites rarely seen in the basement. The granulite xenoliths are thought to represent fragments of the deep crust on account of their high metamorphic grade and their ubiquitous though sparse occurrence in Lesotho kimberlites, and diatremes elsewhere, for example, Uganda (von Knorring and du Bois<sup>1</sup>). This report describes a preliminary investigation into the chemistry of six such granulite xenoliths from Lesotho and comments on the bearing of these results on the evolution of the lower crust. The xenoliths are from the kimberlites at Matsoku (PHN 1646, 2852 and OVKE10303), Mothae (PHN1919) and Lihobong (PHN2588).

## Petrography

The xenoliths possess a distinctive equigranular, granoblastic texture and a limited mineralogy of clinopyroxene, garnet, plagioclase with accessory rutile and mica. The proportions of the minerals vary between samples but are generally in the range of 20–40% each of garnet and clinopyroxene with plagioclase constituting most of the remainder. Optical examination and preliminary microprobe analyses have shown the clinopyroxene to be a jadeitic diopside and the garnet a member of the pyrope–almandine series. Dark green kelyphitic rims around the garnets can attain thicknesses of up to 0.5 mm but are not as regularly developed as those surrounding garnets in ultra-basic xenoliths.

Rutile is the most abundant accessory mineral occurring as discrete grains randomly scattered throughout the rock. Mica, when present, is invariably in the process of decomposition to iron ore set in an aphanitic matrix. Its optical properties suggest that it is an iron rich biotite.

Petrographically, the xenoliths are members of the clinopyroxene–almandine granulite sub-facies<sup>2</sup> or high pressure granulite facies<sup>3</sup>, although the clinopyroxene and garnet compositions do possess some eclogitic affinities. Thus the assemblages must have crystallised under conditions of high temperature and high confining pressure.

## Conditions of xenolith equilibration

As clinopyroxene and garnet are the only ferromagnesian minerals present in the xenoliths, the temperature of crystallisation can be estimated by the application of the geothermometer of Raheim and Green<sup>4</sup>. This is based on the partition

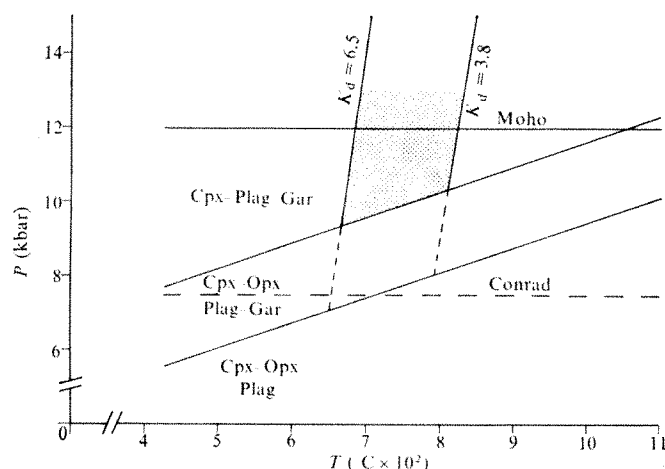


Fig. 1 Pressure, temperature diagram showing isopleths for the range of  $K_d$  values derived from the garnet-clinopyroxene (Gar.-Cpx.) pairs, superimposed upon the relevant phase boundaries for R698 Plag., plagioclase. Opx, orthopyroxene. The pressures corresponding to the depths of the Moho and Conrad discontinuities are shown.

of magnesium and iron between coexisting garnet and clinopyroxene expressed as a distribution coefficient,  $K_d$ . Values of  $K_d$  for five mineral pairs have been calculated from preliminary microprobe analyses, after an empirical correction for ferric iron<sup>5</sup>. The  $K_d$  values fall in the range 3.8–6.5, the isopleths corresponding to these two limiting values are shown in Fig. 1.

The pressure of equilibrium is more difficult to define, the sodium analysis of the pyroxenes is not precise enough to allow the calculation of the molecular proportion of the jadeite end member, previously used as a barometer<sup>6</sup>. Experiments by Irving<sup>7</sup> on pyroxenite and granulite xenoliths of similar compo-

sition allows the definition of approximate pressure limits by extrapolating the observed phase boundaries to the temperatures derived from the  $K_d$  values of the Lesotho xenoliths. It should be emphasised that the pressure estimates are approximations due to the necessarily large extrapolation of the phase boundary from the original data. The experimental runs on a two-pyroxene granulite defined the boundary above which orthopyroxene is unstable, reacting with plagioclase to form garnet and clinopyroxene. By linear extrapolation, this boundary can be used to define the lower limit of equilibration of the Lesotho xenoliths, the latter being of similar composition to the xenolith studied experimentally (see Table 1). The upper pressure limit can only be roughly estimated, as the boundary above which plagioclase is unstable was not defined. Irving's results did, however, reveal a gradual decrease in modal plagioclase with increasing pressure. Although this effect cannot be extrapolated quantitatively to lower temperatures, it seems likely that the same phenomenon would occur in the less extreme conditions. As the Lesotho xenoliths contain relatively high modal abundances of plagioclase (20–40%), it is probable that they equilibrated at pressures not greatly in excess of the minimum pressure defined above.

By application of these constraints, it can be seen (Fig. 1) that the xenoliths probably equilibrated at pressure of 9–13 kbar at temperatures of 660–830 °C. The pressure estimates agree well with those expected at the base of the crust from seismic investigations (22–36 km)<sup>8</sup>. These figures are for the East Transvaal, there being no corresponding figures for Lesotho. The temperature estimates, however, range to high values compared with those expected for cratonic regions. This may be a true reflection of lower crustal conditions or a result of analytical error in the determination of sodium in the pyroxenes, affecting the calculated Fe(III)/Fe(II) ratio and, consequently, the  $K_d$  value.

It is of interest that two-pyroxene, garnet granulite xenoliths of similar bulk composition to the orthopyroxene free varieties have been found in Lesotho kimberlites<sup>9</sup>. According to ex-

Table 1 Major and trace element analyses of seven xenoliths

	PHN1646	PHN1919	PHN2852	OVKF10303	PHN2588	PHN2533	R698
SiO <sub>2</sub>	45.59%	45.26%	47.05%	49.25%	51.24%	50.39%	49.76%
TiO <sub>2</sub>	1.64	1.15	1.01	0.35	0.54	0.70	0.69
Al <sub>2</sub> O <sub>3</sub>	14.40	16.42	17.88	17.44	16.69	15.14	16.83
Fe <sub>2</sub> O <sub>3</sub> *	14.25	11.53	1.47	9.62	10.58	12.16	1.86
FeO			7.71				7.09
MnO	0.21	0.18	0.16	0.15	0.18	0.18	0.17
MgO	9.78	9.86	7.52	10.26	8.60	7.02	8.25
CaO	11.55	8.23	9.59	11.11	8.47	9.05	11.07
Na <sub>2</sub> O	1.65	4.99	3.85	1.86	2.86	3.47	2.78
K <sub>2</sub> O	0.88	0.40	0.93	0.72	1.50	2.08	0.32
P <sub>2</sub> O <sub>5</sub>	0.21	0.10	0.19	0.05	0.05	0.08	0.08
Total	100.14	98.12	99.96†	100.82	100.70	100.27	100.14‡
Ba	3,800 p.p.m.	520 p.p.m.	1,800 p.p.m.	500 p.p.m.	1,700 p.p.m.	3,700 p.p.m.	
Hf	3.4	0.8	0.7	—	—	—	—
Nb	13	4	5	4	2	4	—
Rb	25	14	12	8	23	31	—
Sr	1,050	195	860	1,250	625	700	—
Ta	0.7	0.1	0.1	—	—	—	—
Y	27	13	7	5	4	10	—
Zr	103	26	24	20	21	13	—
Ce	36.7	13.8	7.0	3.9	—	—	ND
Nd	23.6	8.1	6.2	2.8	—	—	ND
Sm	5.6	2.3	1.6	0.8	0.6	—	ND
Eu	1.9	1.2	1.2	0.6	0.7	—	ND
Gd	6.6	2.4	—	—	—	—	ND
Tb	1.0	0.4	0.3	0.2	0.1	—	ND
Tm	0.4	0.2	—	0.2	0.1	—	ND
Yb	3.0	1.3	0.9	0.8	0.7	—	ND
Lu	0.5	0.2	0.2	0.2	0.1	—	ND
K/Rb	292	237	650	747	541	557	—
Ba/Sr	3.6	2.7	2.1	0.4	2.7	5.3	—

Major and trace element whole rock analyses of garnet pyroxene granulite xenoliths from Lesotho Kimberlites. R698, a two pyroxene granulite from Delegate is included for comparison. ND, not determined; —, not detected.

\*Total iron as Fe<sub>2</sub>O<sub>3</sub> (unless FeO is listed).

†Includes H<sub>2</sub>O<sup>+</sup> 2.36%, H<sub>2</sub>O<sup>-</sup> 0.24%. Analysis by O. von Knorring and D. T. Richardson.

‡Includes H<sub>2</sub>O<sup>+</sup> 1.10%, H<sub>2</sub>O<sup>-</sup> 0.14% from ref. 8.

periment, these xenoliths probably equilibrated at lower pressures than the orthopyroxene-free granulites, thus supporting the conclusion that the latter type are derived from near the base of the crust.

### Major and trace element geochemistry

The major element analyses of six xenoliths are given in Table 1 together with trace and rare earth element (REE) data. Also included is an analysis of R698, the two pyroxene granulite used in the experiment<sup>7</sup>. The REE, Ta and Hf were determined by instrumental neutron activation techniques, similar to those described by Gordon *et al.*<sup>10</sup>; the remaining trace elements and the major elements were analysed by X-ray fluorescence.

A notable feature of the major element analyses is their restricted basaltic range, which in terms of alkali and alumina content, is comparable with high alumina and alkali basalts of island arc regimes<sup>11</sup>. The alkaline nature of the xenoliths is a reflection of their high sodium rather than their high potassium contents, although the latter is not particularly depleted. The basaltic composition might suggest that the xenoliths represent pockets of magma that were trapped and solidified at lower crustal depths during a previous magmatic event; an origin that has been suggested for similar xenoliths elsewhere (as at Delegate<sup>7</sup>). The predominantly tholeiitic nature of earlier South African magmatism, however, suggests that this is unlikely as it is difficult to envisage how trapped tholeiitic magma could develop an alkaline and aluminous chemistry.

The narrow basaltic range of the analyses is in marked contrast to the wide compositional variation of granulites of metamorphic terrain<sup>12</sup>. This could be a true reflection of lower crustal chemistry or an artefact caused by the selection of a narrow range of xenolith type. The xenoliths were selected on the basis of texture and mineralogy and no silicic varieties were included as they were not of comparable metamorphic grade. Also the xenoliths are not particularly depleted in  $K_2O$ , a common feature of granulites of metamorphic terrain<sup>13-15</sup>.

The limited major element variation also contrasts with the results of the trace and REE analyses, the latter showing considerable and significant variation between xenoliths. The chondrite normalised abundance diagram (Fig. 2) reveals a variation from xenoliths with light REE enriched patterns and no Eu anomaly (PHN 1646) to those with flatter REE patterns and positive Eu anomalies. One xenolith PHN2588, is thought to possess a light REE depleted pattern as Ce and Nd were not detected. The REE patterns do not particularly resemble those previously observed in other high grade metamorphic rocks (for example, ref. 16). The incompatible elements, Nb, Zr, Hf and Ta, vary comparably with the light REE, being most abundant in PHN1646, less abundant in the intermediate xenoliths and present in undetected amounts in PHN2588. This coherent behaviour of many of the trace elements is only slightly reflected in the small major element variation, such as PHN1646, the xenolith most enriched in the light REE, has a higher  $TiO_2$ , total iron (as  $Fe_2O_3$ ),  $MnO$  and  $CaO$  and a lower  $Al_2O_3$  content than the other xenoliths. With so few analyses, however, it is difficult to assess the significance of this variation.

Before considering the significance of the trace element variation further, it is important to assess the possibility of contamination of the xenoliths by the host kimberlites. Despite stringent precautions taken during sample preparation to avoid inclusion of any carbonated material a comparison of trace element abundances in kimberlite and the xenoliths reveals that selective contamination may have taken place.

Four of the xenoliths possess high K/Rb ratios, in the range 540-747, and are much higher than usual values for kimberlite (about 200)<sup>17</sup>. Such a large difference, together with the grouping of the xenolith values despite the variation in the absolute abundances of the two elements in the xenolith suite, suggests minimal contamination.

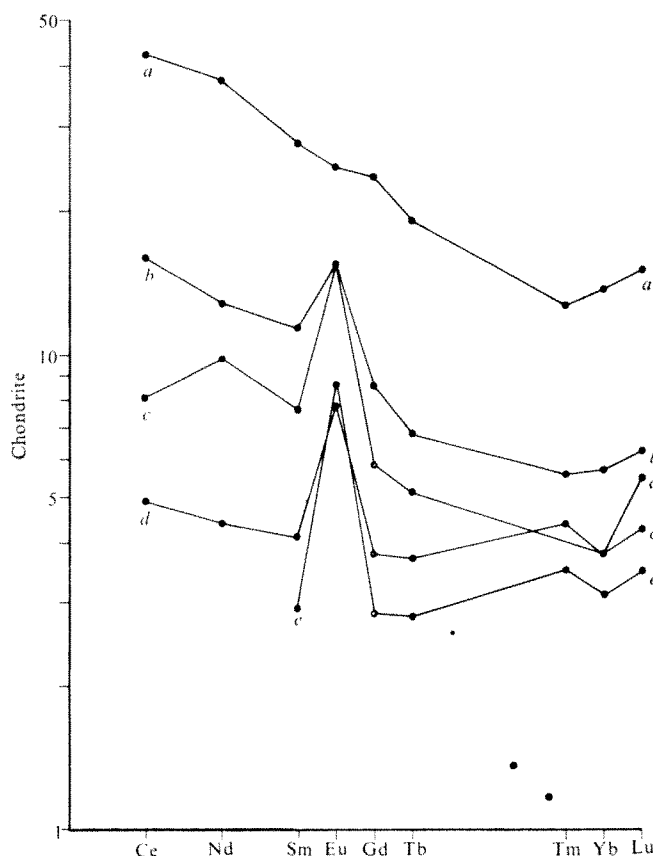
The effect of the kimberlite on the REE abundances of the xenoliths is thought to be minimal as the former rarely exhibit Eu anomalies, a common feature of the xenoliths. If the ob-

served REE variation was a result of contamination of uniformly depleted xenoliths, then it would be expected that the positive Eu anomaly should persist even in the sample most enriched with light REE. As this is not the case, PHN1646 showing no Eu anomaly, it seems unlikely that significant REE contamination has occurred. Similarly, as the other incompatible elements vary coherently with the REE, a lack of significant contamination can be inferred.

In contrast to these conclusions, the concentrations of Ba and Sr and the value of the Ba/Sr ratio each vary over an order of magnitude (see Table 1), generally corresponding to the kimberlite averages of 1,000 p.p.m., 700 p.p.m. and about 1.4 respectively. Also, the two elements behave in a way that is not comparable with the other trace elements. This apparently anomalous variation could be the result of contamination, the elements possibly having been mobilised during eruption by the volatile rich kimberlite.

The basaltic chemistry of the Lesotho granulite xenoliths, and that consequently inferred for the lower crust, contrasts with the observed, more silicic upper layers, in accord with theories suggesting a chemically zoned continental crust<sup>14, 15, 18</sup>. From other experimental results<sup>19</sup>, the existence of a lower crustal layer with a less mafic composition than that inferred from the xenolith analyses has been proposed<sup>19</sup>. The less mafic composition was suggested as, in expected lower crust conditions, normal basaltic compositions take on a plagioclase-free eclogite mineralogy with a density too high to account for the observed seismic velocities. But, systems with high soda and alumina contents, comparable with the xenoliths, allow the stability of plagioclase at much higher pressures, in excess of expected lower crustal conditions. Thus the lower crust maintains a density below that of eclogite while possessing a mafic composition, although it is debatable how the lower crust

Fig. 2 Chondrite normalised relative REE abundance diagram for the five xenoliths analysed. a, PHN 1646; b, PHN1919; c, PHN2582; d, OVKF10303; e, PHN2588. Values for Gd in samples c, d and e are extrapolated, all other points refer to actual analyses.





developed this unusually sodic and aluminous chemistry originally.

Fyfe<sup>20</sup> suggested that crustal zonation resulted from intra-crustal partial fusion of rocks in the upper amphibolite or granulite facies followed by upward migration of the granitic partial melt leaving a relatively infusible and anhydrous residue of pyroxene granulite in the lower crust. In a similar model, Jakes and Taylor<sup>21</sup> proposed that the residue would be plagioclase rich and thereby possess a positive Eu anomaly to balance the large negative anomaly seen in many Andean type igneous rocks that may be equated with Fyfe's granitic partial melt. Initially the xenoliths might seem to fit this model, those samples most depleted in the lithophile and rare earth elements possessing the most pronounced Eu anomalies. It is possible, however, for Eu anomalies to develop in plagioclase rich cumulates and gabbros (such as the Troodos gabbros<sup>22</sup>). Therefore, such an anomaly cannot be regarded as unequivocal evidence for the previous occurrence of partial fusion and may indicate that the xenoliths were originally cumulates or gabbros and that the observed trace and rare earth element variation is the result of trapping different amounts of interstitial liquid in the original rock.

Two notable features of the trace element abundances concern the variation of K, Rb, Y and the heavy REE. Rb, frequently regarded as an incompatible element, does not vary with the other trace incompatible elements. Furthermore, Y and the heavy REE, elements easily accommodated by garnet and, therefore, in the context of garnet bearing assemblages, are compatible, show a similar, though less marked, depletion to the light REE Nb, Zr and so on. These features suggest the stability of a Rb bearing mineral (such as hornblende or biotite) in, and the absence of garnet from, the residuum during the solid-liquid fractionation. Feldspar is also thought to be a stable residual phase from the marked positive Eu anomalies present in all but one of the xenoliths. Hornblende and biotite are unusual minerals to crystallise directly from a basaltic liquid; olivine, plagioclase and pyroxene, are the most common phases to form initially. It, therefore, seems unlikely that the xenoliths are fragments of meta-gabbros or cumulates, but are most likely to be derived from the metamorphosed solid

residuum from the partial fusion of an amphibolite facies assemblage rich in feldspar, hornblende and/or biotite.

Finally, the possibility of metasomatic activity during the evolution of the granulites must be considered. The presence of accessory scapolite has been confirmed in similar granulite xenoliths from Lesotho<sup>9</sup>. This mineral, when found in granulite terrain, has been interpreted as having formed through metasomatic reactions and the addition of volatiles<sup>23</sup>. Furthermore, of those xenoliths analysed, accessory mica is only found in samples PHN1646 and 1919, the two xenoliths most enriched in the light REE. Such an empirical relationship, however, must be further investigated before any firm conclusion can be made. Separate mineral and volatile element analysis should clarify this situation.

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1. von Knorring, O. & du Bois, C. G. B. *Nature* **192**, 1064-1065 (1961).
2. de Waard, D. *Am. J. Sci.* **263**, 455-461 (1965).
3. Green, D. H. & Ringwood, A. E. *Geochim. cosmochim. Acta* **31**, 767-833 (1967).
4. Raheim, A. & Green, D. H. *Contr. Miner. Petrol.* **48**, 179-203 (1974).
5. Ryburn, R. J., Raheim, A. & Green, D. H. *Lithos* **9**, 161-164 (1976).
6. Mysen, B. O. & Heier, K. S. *Contr. Miner. Petrol.* **36**, 73-94 (1972).
7. Irving, A. J. *J. Petrol.* **15**, 1-40 (1974).
8. Hales, A. L. & Sacks, I. S. *Geophys. J. R. astr. Soc.* **2**, 15-33 (1959).
9. Griffin, W. L., Carswell, D. A. & Nixon, P. H. in *Proc. 3rd Int. Kimberlite Conf.* 1977 (in the press).
10. Gordon, G. E., Randle, K., Gole, G. G., Corliss, J. B., Beeson, M. H., & Oxley, S. S. *Geochim. cosmochim. Acta* **32**, 369-396 (1968).
11. Kuno, H. *Bull. Volc.* **29**, 195-222 (1966).
12. Clifford, T. N. *Spec. Pap. Geol. Soc. Am.* No. 156 (1974).
13. Heier, K. S. & Thorensen, K. *Geochim. cosmochim. Acta* **35**, 89-99 (1970).
14. Lambert, I. B. & Heier, K. S. *Geochim. cosmochim. Acta* **31**, 377-390 (1967).
15. Lambert, I. B. & Heier, K. S. *Lithos* **1**, 30-43 (1968).
16. Green, T. H., Brunfelt, A. O. & Heier, K. S. *Geochim. cosmochim. Acta* **36**, 241-257 (1972).
17. Harris, P. G. & Middlemost, E. A. K. *Lithos* **3**, 77-88 (1970).
18. Shaw, D. M. *Geochim. cosmochim. Acta* **32**, 573-602 (1968).
19. Green, T. H. *Phys. Earth Planet. Int.* **3**, 441-451 (1970).
20. Fyfe, W. S. *Geol. J. Spec. Issue* No. 2, 201-216 (1970).
21. Jakes, P. & Taylor, S. R. *Geochim. cosmochim. Acta* **38**, 739-745 (1974).
22. Kay, R. W. & Senechal, R. G. *J. geophys. Res.* **81**, 964-969 (1976).
23. Edwards, A. B. & Baker, G. J. *geol. Soc., Austr.* **1**, 1-32 (1953).

## Geology and palaeontology of Neogene strata of Pakistan

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*A joint study of the Potwar Plateau of Pakistan is yielding abundant material for stratigraphic and palaeontological reassessment.*

THE Siwalik rocks of the Indo-Pakistan subcontinent have interested scholars for more than a century<sup>1,2</sup>, in particular because of the presence in Siwalik beds of hominoid primates. *Ramapithecus punjabicus*, first described from India but known best from what are now quite large samples from Pakistan, is widely believed to be the earliest recognisable hominid or human

ancestor. At least three other hominoid species are known from the Siwaliks. An abundant associated vertebrate fauna is known from Siwalik rocks, spanning the time between 13 and 1 Myr ago. This period is believed to record the time of differentiation of Hominidae, and document the first radiation of higher primates into non-forest habitats. These rocks and their contained faunas figure widely in schemes of primate evolution, mammalian biostratigraphy and South Asian geology, and are increasingly important for the study of Asian climatic and tectonic history.

Since 1973, collaborative research between the Geological Survey of Pakistan (GSP) and the Peabody Museum of Yale University (YPM) has been in progress in the Potwar Plateau of

Pakistan. We give here a preliminary account of the stratigraphy and correlation of important areas within the Potwar Plateau. The results reported are tentative and subject to change because much detailed work remains to be done. Nonetheless we present them as working hypotheses to geologists, palaeontologists and palaeoanthropologists.

### Potwar geology

The Potwar Plateau of the Punjab province (72° 30' E, 33° 00' N) is an elevated area of some 20,000 km<sup>2</sup> bounded to the north by the Kala Chitta and Margala Hills, south by the Salt Range, east by the Jhelum River and west by the Indus River. Neogene molasse was deposited in subsiding basins on the southern flanks of the rising Himalayas. During and after deposition the area was subject to folding and faulting. A good deal of the Plateau is covered by late Pleistocene alluvium, but substantial amounts of Neogene rocks are exposed as badlands. Of particular importance, Siwalik sediments form the Soan synclinorium, the axis of which runs roughly east-west, and are also exposed in anticlinal and monoclinical belts along its northern and southern margins.

Siwalik rocks in the Potwar Plateau were first subdivided into 'Lower', 'Middle' and 'Upper' units by Pilgrim in 1910 (ref. 3). The subdivisions were then based on both lithological and palaeontological criteria, and clearly were meant to have temporal significance. Until recently, rock, faunal and temporal criteria and definitions have been entwined in discussions of Siwalik stratigraphy, causing confusion. In 1913 Pilgrim<sup>1</sup> subdivided the Middle Siwaliks into Dhok Pathan beds and included fauna, making the Dhok Pathan zone, and an underlying Nagri zone. The Lower Siwalik unit was described as the Chinji zone, while Tatrot and Pinjor zones were defined for the Upper Siwaliks. Pilgrim's 'zones' have something of the status of stages (time-rock units) of current stratigraphic usage. In theory, the units were intended to be bounded by isochronous surfaces. Lithology, however, was frequently used together with fauna in classification as though similar rock-type implied similar time. It is now known that because of facies change, gross lithology alone is a poor correlative tool; Pilgrim (ref. 3, page 186) noted this, although he often ignored its implications.

Anderson in 1927 used the terms 'Chinji stage', 'Middle Siwalik' and 'Upper Siwalik' solely in a lithological sense, and included discussions of thickness and facies variation. Essentially his concepts are 'formational' in current stratigraphic usage<sup>5</sup>; however, his 'Middle' and 'Upper Siwalik' categories are different from those of most other workers.

In the early 1930s Cotter and Colbert published slightly different stratigraphic schemes for the Siwaliks. Cotter<sup>6</sup> used a 'stage' terminology ('Chinji Stage' and so on), the stages being defined on the basis of lithology and fauna. Colbert<sup>2</sup> subdivided the sequence into 'zones' using the same terminology, based almost exclusively on faunas. Lewis<sup>7</sup> used a 1933 North American stratigraphic committee report in defining formations for the Siwalik subdivisions. Following Pilgrim, Anderson, Cotter and others he defined Tatrot, Dhok Pathan, Nagri, Chinji and Kamliak Formations, giving type localities and very brief lithological descriptions. But he also included descriptions of faunas as part of the definitions, and it seems that the units are stages of current usage, rather than formations. Gill<sup>8</sup> used a 'stage' terminology for concepts that are basically formational.

In 1973 the Stratigraphic Committee of Pakistan<sup>9</sup> formally defined formations solely on lithological criteria. The nomenclature used (Chinji, Nagri and so on) was that introduced by Pilgrim 60 yr earlier on the basis mainly of faunas, and which has since been used by many workers in various ways as rock and time-rock units. The use of these identical parallel terminologies has proved extremely confusing. We agree with Fatmi<sup>9</sup> that Pilgrim's names should now be confined to properly defined lithological units. The formation names should not be used outside the type areas and 'mappably' contiguous areas. A new nomenclature for biostratigraphic and time-stratigraphic units is thus necessary.

Siwalik deposition is mainly fluvial and cyclic<sup>10-12</sup>. Typical exposures are characterised by repetitive units, commencing with

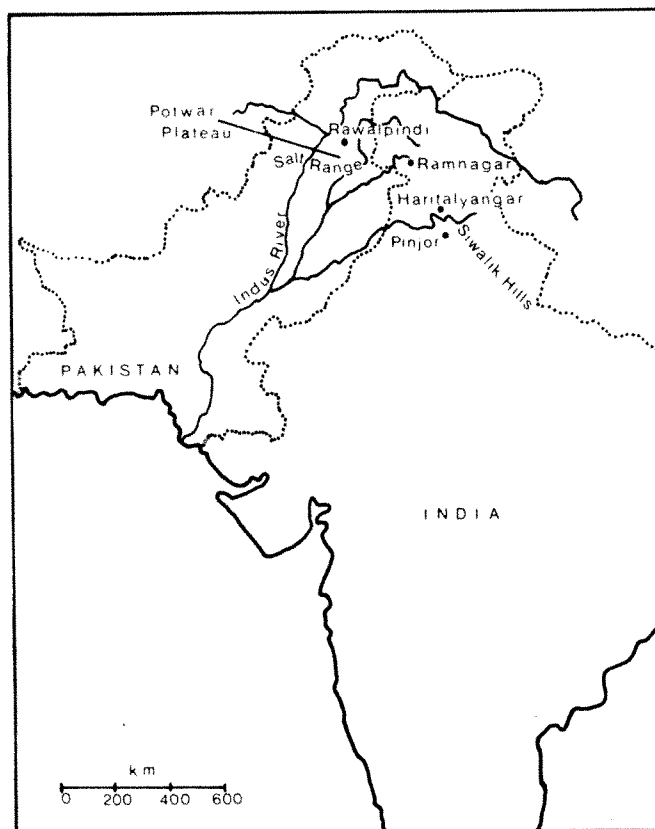


Fig. 1 Map of South Asia showing position of Potwar Plateau.

single sandstone bodies grading upwards into fine-grained silts and clays which are in turn abruptly truncated by the sandstone of the succeeding cycle<sup>10</sup>. The sandstones probably represent lateral accretion or point-bar, channel lag, cut and fill and channel splay deposits, the finer sediments being vertical accretion or overbank deposits which may preserve palaeosols. Other exposures are characterised by sequences almost lacking fine-grained sediments, while multistoried sandstone attain considerable thickness. As a whole, the beds imply a dominantly braided fluvial regime and a relatively rapidly aggrading multiple-channelled river.

The Chinji Formation (72° 22' E, 32° 41' N) is defined<sup>9</sup> south of Chinji Village on the southern margin of the Soan synclinorium, and consists of a series of sandstones separated by thick red silts. The succeeding Nagri Formation (72° 30' E, 32° 46' N), defined north-east of Chinji Village near Sethi Nagri<sup>9</sup>, is made up of more massive sandstones with only minor overbank episodes.

Unfortunately the succeeding Dhok Pathan Formation<sup>9</sup> is not defined in the same area as the Nagri Formation. Its type locality is at Dhok Pathan (72° 31' E, 33° 8' N) on the northern flank of the Soan synclinorium, some 45 km north of Chinji Village and 40 km north and west of the Nagri type locality in the Gorge of the Gambhir River near Sethi Nagri. The Dhok Pathan Formation is, like the Chinji, predominantly argillaceous, although the overbank deposits of the former rarely match the rich red hues of the latter. Some nine fluvial cycles are recorded by us at Dhok Pathan village, where the formation is unconformably overlain by the much younger, horizontally bedded Potwar Silts.

We assume that the top of the Nagri Formation, as defined in the south in its type locality by transition from mainly arenaceous to mainly argillaceous beds, is time-equivalent to the similarly defined base of the Dhok Pathan Formation in the north. This may not be the case, however. It has been long realised, though much neglected, that these deposits are hardly atypical among fluvial sediments in showing significant lateral variation in thickness and lithology, often over quite short distances<sup>4,8,13</sup>.

Detailed mapping and section measuring in these three stratotype areas should be completed within the next 2 yr. In addition



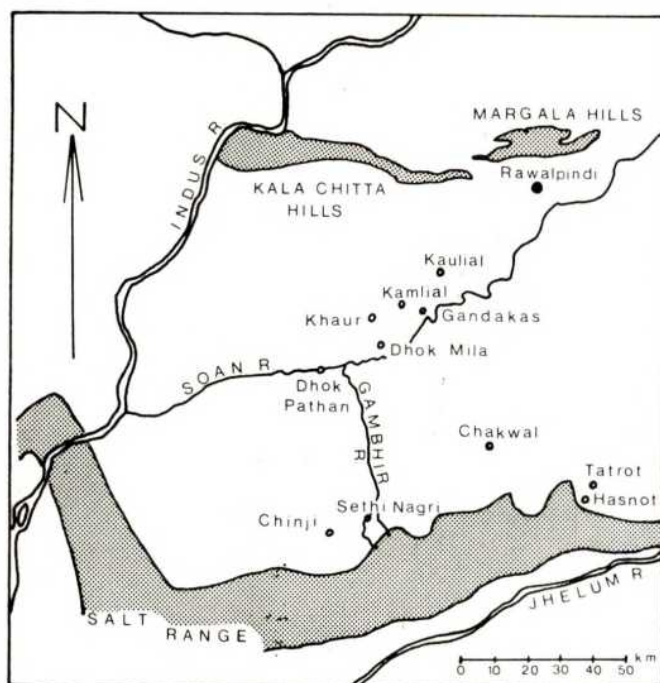


Fig. 2 Map of Potwar Plateau showing main fossil collecting areas.

we have explored in detail northern exposures to the east and north of Dhok Pathan and south of Khaul, near the villages of Dhok Mila, Dhok Ganja, Gandakas and Kaulial ( $72^{\circ}30' E$  to  $72^{\circ}45'$ ,  $33^{\circ}9' N$  to  $33^{\circ}22' N$ ). Major sandstone units have been strike-mapped north-east into this area from the Dhok Pathan type area, and it is clear that there are major lithofacies changes from west to east. Thus, as noted, at Dhok Pathan village, the Dhok Pathan Formation consists of some nine fluvial cycles, with overbank sediments dominant. The underlying beds presumed laterally equivalent to the Nagri Formation are arenaceous, consisting mainly of massive multistoried sandstones. Some 25 km to the north-east near Gandakas the same nine fluvial units are present in the Dhok Pathan Formation; however, near Gandakas the upper two-fifths of the underlying (presumed) Nagri Formation are argillaceous and lithologically similar to the Dhok Pathan Formation.

The Upper Siwalik Tatrot Formation<sup>7</sup> is defined 85 km to the east of Chinji in a basin centred around the village of Hasnot ( $73^{\circ}21' E$ ,  $32^{\circ}52'30'' N$ ). The Tatrot Formation (not recognised by Fatmi) rests unconformably on sediments of presumed Middle and Lower Siwalik age. Although the formational names 'Dhok Pathan', 'Nagri' and 'Chinji' have been given to rocks in this area they have not yet been mapped into the type areas; caution should be exercised until this has been effected.

Mapping is to continue in each of these areas, in collaboration with an intensive programme to establish a regional magnetic polarity stratigraphy being undertaken by us and by a group from Peshawar University, Dartmouth College, the Lamont-Doherty Geological Observatory and the University of Arizona. Such studies, together with a continuing search for radiometrically datable sediments, must be completed before adequate temporal correlations between the various areas can be made. We assume that major sandstone units used in strike mapping (between, for example, the villages of Dhok Pathan and Gandakas) are minimally time-transgressive, at least for purposes of local correlation of middle and late Miocene sequences of strata.

Extensive faunas were collected in the late nineteenth and early twentieth centuries from the Potwar Plateau, principally from the Chinji, Dhok Pathan and Hasnot areas; a small fauna was also obtained from Sethi Nagri. These collections, together with those from Haritalyangar in India, 425 km to the south-east, and Ramnagar in Kashmir, 250 km to the east, formed the basis for

Table 1 Vertebrate faunas from the Chinji Formation

Mammalia	Artiodactyla
Primates	<i>Listriodon pentapotamiae</i>
<i>Sivapithecus sivalensis</i>	<i>Conohyus chinjiensis</i>
<i>S. indicus</i>	<i>Lophochoeerus</i> sp.
<i>Ramapithecus punjabicus</i>	<i>Merycopotamus pusillus</i>
Creodonta	Tragulidae spp.
<i>Hyainailouros bugtiensis</i>	<i>Giraffokeryx</i> sp.
<i>Dissopsalis carnifex</i>	<i>Protragocerus gluten</i>
Carnivora	<i>Miotragocerus gradiens</i>
? <i>Viverra chinjiensis</i>	<i>Kubanostragus sokolovi</i>
Hyaenid indet.	? <i>Pseudotragus potwaricus</i>
<i>Percrocuta carnifex</i>	<i>Sivoreas eremita</i>
<i>Miohyaena</i> cf. <i>montadai</i>	<i>Gazella</i> sp.
' <i>Sivasmilus</i> '	Tubulidentata
(= <i>Paramachaerodus</i> ) <i>copei</i>	<i>Orycteropus</i>
<i>Sivaelurus chinjiensis</i>	Rodentia
Felid indet. (very small)	cf. <i>Rhizomyidae</i> indet.
? <i>Sansanosmilus</i> sp.	<i>Copenys</i> sp.
<i>Martes lydekkeri</i>	<i>Megacricetodon</i> sp.
? <i>Martes</i> sp.	<i>Antemus chinjiensis</i>
<i>Vishnuonyx chinjiensis</i>	Reptilia
Mustelinae sp.	Chelonia
Amphicyoninae (large sp.)	<i>Trionyx</i> sp.
<i>Amphicyon</i> sp.	<i>Lissemys</i> sp.
<i>Vishnucyon chinjiensis</i>	Ophidia
Perissodactyla	<i>Acrochordus</i> sp.
<i>Chalicotherium salinum</i>	Colubridae sp.
Rhinocerotidae spp.	Boidae indet.

the biostratigraphic zonation developed by Pilgrim and elaborated by many others<sup>1,2,7,14-20</sup>.

The 'Chinji fauna' was based on material mainly from Chinji, and also on fossils from Ramnagar in Kashmir, from rocks of similar lithology (presumed at the time therefore to be of similar age). 'Nagri faunas' were partly based on the fossil mammals from Sethi Nagri, north-east of Chinji, but predominantly on specimens from sediments more than 400 km away at Haritalyangar with which they were correlated on very slender evidence. The bulk of the 'Dhok Pathan fauna' came not from Dhok Pathan itself but from around Hasnot, 90 km to the south-east on the opposite margin of the Soan synclinerium. 'Tatrot faunas' came from Tatrot and Kotal Kund near Hasnot, and from Bhaun, 60 km to the west. Given that each 'fauna' is geographically heterogeneous, often markedly so, and that faunas from different areas were frequently 'lumped' on the basis of lithological criteria now known to be of dubious temporal value, one might well suspect that problems could develop; we aim here to clarify at least some of these.

The bulk of earlier collections from Chinji came mostly from the middle third of the Chinji Formation. Subsequently, collections by Brown, Lewis, Dehm and von Koenigswald, as well as the present project, sampled the upper parts of the formation. The Nagri type fauna comes from Nagri (presumably one of the villages of Sethi Nagri), north-east of Chinji. According to Pilgrim<sup>1</sup>, the type locality for the faunal assemblage is 1,500 feet above the top of the Chinji Formation, some three-fifths of the way up the Nagri Formation in this area. What are probably very close or identical localities have been collected by Dehm, von Koenigswald and ourselves (GSP-YPM locality 311). At Dhok Pathan, we determined that the bulk of the classic fauna comes from the upper half of the formation, the lower portion of which is covered by the Soan River.

In the newly prospected regions to the east of Dhok Pathan we have collected fossils from over 30 fluvial cycles, nine of which we equate by strike mapping with the Dhok Pathan Formation at Dhok Pathan; the underlying cycles correlate with the upper part of the Nagri Formation, spanning approximately its top half. Within the Nagri Formation in the Dhok Mila-Gandakas-Kaulial area, we have informally designated the top few cycles the 'Utran unit', and the immediately underlying series, the 'Dhok Mila'. The upper four cycles of the Dhok Pathan Formation we have informally termed the 'Kundvali unit' while the lower five form the 'Gandakas unit'. On the basis of both relative stratigraphic position and close faunal similarity, we tentatively



**Table 2** Vertebrate faunas from the Nagri Formation

Mammalia	<i>Merycopotamus nanus</i>
Insectivora	<i>M. dissimilis</i>
Soricidae indet.	<i>Dorcabune nagrii</i>
Primates	<i>Dorcatherium majus</i>
?Lorisidae indet.	<i>D. minus</i>
<i>Sivapithecus sivalensis</i>	cf. <i>Dorcatherium</i> sp.
<i>S. indicus</i>	cf. <i>Sivatherium</i> sp.
<i>Ramapithecus punjabicus</i>	<i>Gazella</i> sp.
cf. <i>Gigantopithecus</i> sp.	<i>Miotragocerus punjabicus</i>
Creodonta	<i>Selenoportax vexillarius</i>
cf. <i>Isohyaenodon</i>	? <i>Pseudotragus</i> sp.
Carnivora	<i>Boselaphini</i> very small n. gen., n. sp.
Viverrinae 2 sp.	Rodentia
? <i>Herpestinae</i> sp.	<i>Sciuridae</i> indet.
? <i>Progenetta</i> sp.	<i>Gliridae</i> indet.
<i>Paliyaena sivalense</i>	<i>Rhizomyoides</i> sp.
? <i>Miohyaena</i> n. sp.	<i>Kanisamys sivalensis</i>
<i>Percrocuta carnifex</i>	<i>Progonomys</i> n. sp.
<i>Percrocuta grandis</i>	<i>Parapodemus</i> sp.
? <i>Sivaclurus</i> sp.	cf. ' <i>Mastomys</i> ' <i>colberti</i>
<i>Machairodontinae</i>	Reptilia
? <i>Martes</i> sp.	Chelonina
<i>Mustelinae</i> sp.	<i>Testudo</i> sp.
<i>Eomellivora</i> sp.	<i>Emydidae</i> sp. incl. <i>Kachuga</i> sp.
<i>Sivaonyx bathygnathus</i>	<i>Geomyda</i> sp.
<i>Amphicyon</i> sp.	<i>Trionyx</i> sp.
Proboscidea	<i>Lissemys</i> sp.
<i>Deinotherium</i> sp.	<i>Crocodylia</i>
<i>Gomphotheriidae</i> indet.	<i>Crocodylus palaeindicus</i>
<i>Perissodactyla</i>	<i>Gavialis hysudricus</i>
<i>Hipparion</i> small and large spp.	<i>Ophidia</i>
<i>Chalicotherium</i> cf. <i>salinum</i>	<i>Python</i> sp.
<i>Artiodactyla</i>	<i>Acrochordus</i> sp.
<i>Propotamochoerus hysudricus</i>	<i>Colubridae</i> indet.
<i>Propotamochoerus</i> sp.	<i>Lacertilia</i>
<i>Conohyus</i> sp.	<i>Varanus</i> sp.
<i>Tetraconodon</i> sp.	Amphibia
<i>Hippopotamodon sivalense</i>	Anura indet.
(= <i>Dicoryphochoerus titan</i> )	Mollusca
<i>Schizochocerus</i> sp.	Bivalvia
	Unionidae indet.
	Gastropoda indet.

correlate the southern Nagri faunas ('Nagri'/Sethi Nagri)/locality 311) with those from the middle of the Nagri Formation in the north.

Thus in the region of the stratotypes and 'mappably' adjacent areas we have collected faunal remains from most of the Chinji Formation, especially the middle and upper portions, and from a continuous sequence consisting of the upper half of the Nagri Formation and the entire Dhok Pathan Formation. These two faunal blocks are separated by a considerable stratigraphic, and probably temporal, gap which is only sparsely fossiliferous because of the predominantly arenaceous sedimentation of the lower half of the Nagri Formation in the type locality and adjacent areas.

### New faunal analyses

About 13,000 catalogued specimens from approximately 350 localities have been recovered by the GSP-YPM expedition; these sample a diverse fauna of mammals, lower vertebrates and invertebrates. Most major groups are undergoing taxonomic revision. Previous collections in the British Museum (Natural History), Indian Museum, American Museum of Natural History, Yale Peabody Museum, Munich Geological Institute, and Geological Institute, University of Utrecht, are also being studied. In many cases it has been possible to determine quite closely the stratigraphic positions of previously collected specimens. Significant new material has been recovered, particularly primates, bovids, suids, carnivores and rodents (several hundred specimens), which should help considerably in biostratigraphic and other studies.

The fauna from the Chinji area contrasts markedly with that from Nagri and the area around Khaur; the latter, in contrast, apparently differs only minimally from that collected in the Dhok

Pathan Formation at Dhok Pathan and south of Khaur. The following faunal lists, based on our own collections with good stratigraphic control, or on previous material of known provenance, should be regarded as preliminary, although we feel that in broad outline this account is likely to stand. More detailed studies will follow as collections are expanded and major revisions are completed.

### Chinji Formation

The vertebrate faunas (Table 1) have been collected from the upper two-thirds of the Chinji Formation in the type area (directly south of Chinji Village) and from some 10 km east and west of Chinji. Many other vertebrates and invertebrates have been collected from Chinji, and some have been recently revised<sup>21-24</sup>. Others remain to be restudied.

Among several thousand specimens, we have collected neither cervids nor *Hipparion* from the formation. Rodents from surface collections reviewed by Black<sup>25</sup>, with the possible exception of a rhizomyid, have also not been recovered by us. Overall faunal resemblances are to Astaracian<sup>26</sup> faunas of Europe and west Asia, and are especially marked among the suids, *Kubanostragus* (known elsewhere from Belometscheskaia in the Caucasus, Gabounia, ref. 27), and the cricetids, which resemble those from Europe. The murid is a new primitive species, possibly ancestral to later murids<sup>36</sup>. There are a few resemblances to African faunas (Fort Ternan, Ngorora), presumably reflecting an earlier period of African-south Asian faunal connections, although they are not marked. An age of between 11 and 13 Myr is suggested for the Chinji Formation and its fossils based on biostratigraphic correlations to geochronologically controlled sequences elsewhere in the Old World<sup>28</sup>.

### Nagri Formation

The bulk of the mammals recovered by us (Table 2) come from the four fluvial cycles of the Dhok Mila unit south of Khaur, although some material is known from the uppermost Utran unit and from Sethi Nagri. At present, this is our best sampled unit. Other material remains to be studied and revised.

This fauna, the bulk of which comes from a relatively restricted stratigraphic range in the northern margin of the syncline, is clearly different from that of the Chinji area. This is to be expected considering the rather substantial thickness of Nagri Formation separating the two faunas; unfortunately this intervening section is only patchily fossiliferous. The fauna is most closely comparable with those from Eurasian rather than African sites. The bovids (especially *Miotragocerus punjabicus*), suids, rodents (*Progonomys* and *Parapodemus*) and the two species (compare ref. 29) of *Hipparion* suggest a correlation with late Vallesian or early Turolian faunas. The fauna from Samos, with an age of about 9 Myr (ref. 30), and Turkish faunas, aged between 9 and 10 Myr (ref. 31), are probably comparable in age with those from the upper part of the Nagri Formation.

Faunal resemblances, especially of suids and bovids, and preliminary geological mapping suggest that the southern Nagri Formation site(s) at Sethi Nagri sampled by Pilgrim, Dehm, von Koenigswald, and GSP-YPM locality 311 are very similar in age to those in the north.

The first appearance of *Hipparion*, as documented by us, falls in the lowest or 'Pari unit' (our informal usage) of the Nagri Formation. This suggests that much of the lower half of the Formation will prove to contain faunas of earlier Vallesian type.

### Dhok Pathan Formation

An extensive fauna is known from the formation, both around Dhok Pathan Village and to the south and east of Khaur. Much of the earlier collected material has been located relative to our stratigraphic columns. The classic 'Dhok Pathan' fauna from the type area of the formation is known mainly from its upper half, informally termed the Kundvali unit by us. To the east, lower fossiliferous levels (Gandakas unit) have now been collected so that a continuous column has been sampled from sediments equivalent to the upper portions of the Nagri Formation through

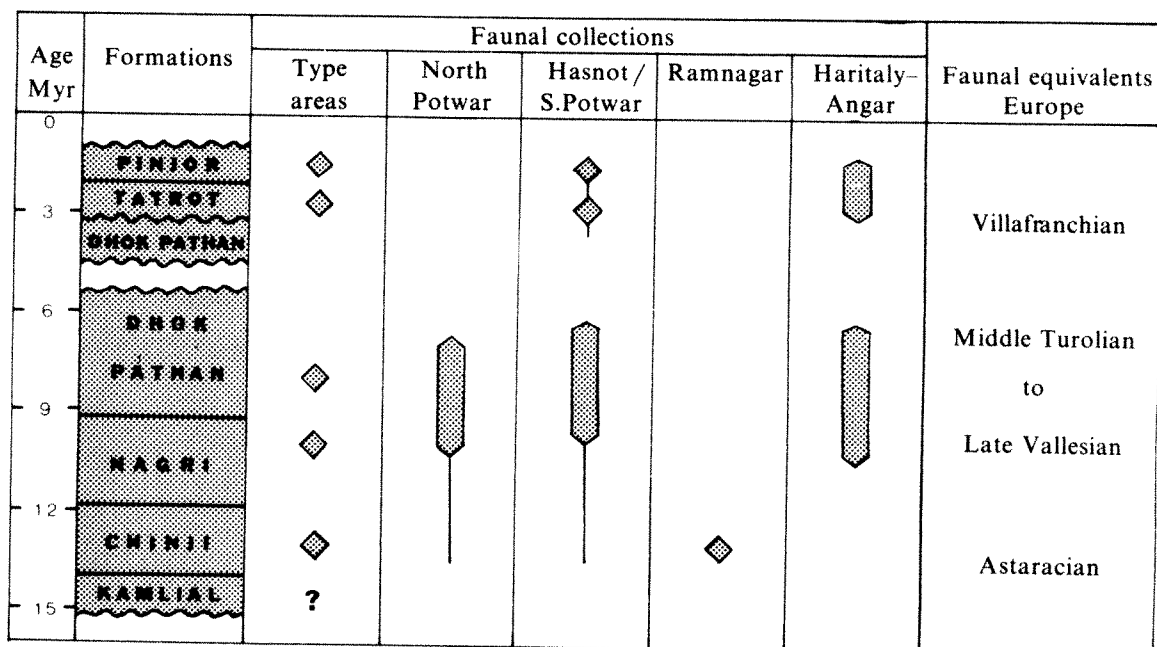


Fig. 3 Tentative stratigraphic chart showing Siwalik formations, main faunal collections and faunal comparisons.

to the upper levels of the Dhok Pathan Formation at Dhok Pathan.

Dhok Pathan Formation faunas are, for the most part, very similar to those of the underlying sediments; thus the suids, carnivores and bovids continue virtually unchanged. High in the Dhok Pathan Formation, however, between local sandstones 6 and 8 of our terminology, there are several interesting faunal changes. *Miotragocerus* and other bovids apparently become extinct; Reduncini make their first appearance (known elsewhere from Kenya in the Mpesida Beds and Lukeino Formation of the Baringo Basin dated at 7 and 6.5 Myr respectively<sup>32</sup>), together with a species very similar to *Prostrepsiceros houtumshindleri* from Maragha in Iran, and a species of cf. *Sus*. Overall, the Dhok Pathan Formation faunas resemble those from the Turolian Land Mammal 'Age' as defined in Europe, North Africa and West Asia. A suggestion of absolute age can be obtained from radiometric dates on the Samos faunas of around 9 Myr (ref. 30), and of around 8 to 9 Myr (or perhaps less) on similar Turkish faunas<sup>31</sup>.

Of interest is the overall similarity between the faunas of the upper Nagri Formation and its equivalents and those from the Dhok Pathan Formation. Their probably rather restricted time range seems to fall close to the boundary between Vallesian and Turolian Land Mammal 'Ages'. Only at the top of the Dhok Pathan Formation is there evidence of some faunal change. It has long been assumed, however, that the 'Nagri' and 'Dhok Pathan' faunas were rather different, and it is worth considering why this view should have arisen, given the relative similarity of our collections from the type areas of the formations. As Pilgrim<sup>1,3</sup> outlined, the 'Dhok Pathan fauna' contained specimens from around Dhok Pathan itself, as well as from Niki (or Nikki) and Hasnot. Hasnot, from which a substantial fauna is known, is in a relatively isolated part of the southern Potwar Plateau, 90 km from Dhok Pathan. A close inspection of the works of Pilgrim and in particular of Colbert<sup>2</sup> shows that, although a number of species are common to both the Dhok Pathan and Hasnot areas, most of the species of the 'Dhok Pathan fauna' that differ from those from the upper parts of the Nagri Formation both at Nagri and near Khaur, are in fact known mainly or exclusively from around Hasnot.

The supposed 'Middle Siwalik' deposits at Hasnot have yielded several species of Reduncini, cervids, several suids including *Sivachoerus*, *Hippohyus*, *Sivahyus* and *Sus*, the oldest Siwalik cercopithecoid *Presbytis sivalensis*, and a rodent fauna clearly distinct from that found in the upper part of the Nagri Formation and its equivalents.

Probably the deposits at Hasnot include a succession of rocks

of similar lithology to those at Dhok Pathan that is the same age as and younger than the type Dhok Pathan Formation. Rocks of similar age, so far unexplored, are probably present in the area east of Kaulial.

### Potwar biochronology

The improper extension of formational names to lithological sequences that are not in mapped continuity with the type areas, especially in fluvial deposits where rapid changes in facies are common, cannot be too strongly condemned. Ultimately, we hope that correlations between the principal Potwar fossiliferous areas can be documented using non-faunal methods—strike-mapping, magnetic polarity stratigraphy and radiometric age determinations. Such efforts will be continued by both our group and that from Peshawar-Dartmouth-Lamont-Arizona. Until that time we believe that the biostratigraphic scheme for the Potwar Plateau outlined here will serve as a reasonable working hypothesis. Ultimately, an improved biostratigraphic zonation with names different from formational ones must be established.

Detailed lithostratigraphic and biostratigraphic work in the central Potwar region enables us tentatively to isolate two rock sequences (and time periods) for which faunas can be described. The earlier of these comes from the upper two-thirds of the Chinji Formation at its type locality, and most closely resembles Eurasian and African Astaracian and Astaracian-equivalent faunas, ranging in age from perhaps 11 or 12 to 13 or 14 Myr. The later faunal sequence spans the upper half of the Nagri Formation and equivalent rocks and almost the entire superposed Dhok Pathan Formation, comprising material from Nagri itself, Dhok Pathan and the region south and east of Khaur contiguous with the Dhok Pathan area. These faunas probably span the period between about 10 Myr and perhaps 7.5 or 8 Myr, on the basis of comparisons with other dated Eurasian and African faunas. Within this faunal sequence little change can be observed, although some (apparent) extinctions and appearances are recorded high in the Dhok Pathan Formation. A preliminary interpretation of faunas from the upper parts of the so-called Middle Siwaliks at Hasnot suggests that at least part of the fauna there is younger than the bulk of that from Dhok Pathan; an average age of around 7 Myr can be tentatively suggested for these younger elements.

The period between the Chinji Formation and upper parts of the Nagri Formation and equivalents is represented by poorly fossiliferous sandstones. Known faunas from these formations and equivalents are substantially different. Within the poorly

fossiliferous part of the column we locate the first appearance of *Hipparion*, in the basal 'Pari unit' of the Nagri Formation. Other workers have reported *Hipparion* from the Chinji Formation<sup>1,2,6</sup>, but the provenance of these finds can be questioned<sup>3,3</sup> or they consist of a very few isolated teeth which might well not have been *in situ*. In four seasons we have not located *Hipparion* in the Chinji Formation, and in our opinion this equid is an immigrant arriving after deposition of the Chinji Formation.

### Other Siwalik faunas

Two other areas outside the Potwar Plateau yield abundant Miocene fossils, including primates—the regions around Ramnagar and Haritalyangar. Material from Ramnagar, 100 km north-east of Jammu in Kashmir, is apparently similar to faunas from the type Chinji Formation. Mammals include *Listriodon* and other characteristic species, while *Hipparion* is absent<sup>16</sup>. Around Haritalyangar, Siwalik rocks are extensively exposed<sup>1</sup>. The terms 'Chinji', 'Nagri' and so on have been widely used both for formations and faunas in these areas, in our view unjustifiably. At Haritalyangar the major Miocene fossiliferous horizons are exposed as a sequence of predominantly argillaceous beds in the 'Lower' and 'Upper Alternations'; these comprise almost 70 fluvial cycles<sup>11,12</sup>. According to Pilgrim<sup>1</sup> and to recent work by teams from Chandigarh and Yale Universities, *Hipparion* is found throughout the section. But most of the fauna from the Haritalyangar region, including that used by Pilgrim to make up the bulk of his 'Nagri fauna', as well as that collected later by Lewis<sup>19</sup>, Prasad<sup>34,35</sup> and the Chandigarh-Yale group, probably comes from a relatively restricted zone of less than 10 fluvial cycles, 20 or more cycles above the base of the Lower Alternations (with *Hipparion*). Recent analysis of faunas from Haritalyangar in Calcutta and at Yale (Lewis collections) by M.P., H.T. and L.L.J. suggests a close similarity of bovids, rodents and suids to those from the upper part of the Nagri Formation of the central Potwar Plateau. Such a correlation, however, needs to be confirmed independently, and is probably best done magneto-stratigraphically.

If correct these correlations suggest that the bulk of known Indo-Pakistan Lower and Middle Siwalik faunas fall into two groups: an earlier one (material from Chinji and Ramnagar) around 12 Myr old, and a younger one (Nagri, Dhok Pathan, Khaur, upper Hasnot, Haritalyangar) 10–7 Myr old. Some material from Khaur-Dhok Pathan, Hasnot and Haritalyangar probably fills the gap between the two groups, though only sparsely. This has important implications for faunal studies,

especially those of the hominoid primates, because some Lower Siwalik mammals may be almost twice as old as some from the Middle Siwaliks.

We have recovered almost 90 new hominoid primates during the past three seasons, mostly from the Khaur area. They are from a relatively restricted stratigraphic range, mostly from the Dhok Mila unit in the upper third of Nagri Formation equivalent strata. Some other material comes from Sethi Nagri and is probably of similar age, as estimated from faunal associations. The material seems to be sampled from at least three species, *Ramapithecus punjabicus*, *Sivapithecus indicus* and one similar to *Gigantopithecus*. Some of the specimens have relatively complete jaws, and also include the first hominoid postcranial remains from the Siwaliks. They are the subject of the following report.

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- Pilgrim, G. E. *Rec. Geol. Surv. India* **43**, 264 (1913).
- Colbert, E. H. *Trans. Am. Phil. Soc.* **27**, 1 (1935).
- Pilgrim, G. E. *Rec. Geol. Surv. India* **40**, 185 (1910).
- Anderson, R. V. V. *Bull. Geol. Soc. Am.* **38**, 665 (1927).
- Krumbein, W. C. & Sloss, L. L. *Stratigraphy and Sedimentation* (Freeman, New York, 1951).
- Cotter, G. *Mem. Geol. Surv. India* **55**, no. 2 (1933).
- Lewis, G. E. *Am. J. Sci.* **33**, 191 (1937).
- Gill, W. D. Q. *J. Geol. Soc., Lond.* **107**, 375 (1952).
- Fatmi, A. N. *Mem. Geol. Surv. Pakistan* **10**, 1 (1973).
- Duff, P., Hallam, A. & Walton, E. *Cyclic Sedimentation* (Elsevier, Amsterdam, 1967).
- Johnson, G. D. *Neogene Molasse Sedimentation in a Portion of the Punjab* (University Microfilms, Ann Arbor, 1971).
- Johnson, G. D. *Geol. Rundsch.* **66**, 192 (1977).
- Morris, T. O. Q. *J. Geol. Soc., Lond.* **94**, 385 (1938).
- Pilgrim, G. E. *Pal. Ind. NS* **8**, 1 (1926).
- Pilgrim, G. E. *Pal. Ind. NS*, **18**, 1 (1932).
- Pilgrim, G. E. *Am. Mus. Novit.* **704** (1934).
- Pilgrim, G. E. *Mem. Geol. Surv. India* **26**, 1 (1939).
- Matthew, W. D. *Bull. Am. Mus. Nat. Hist.* **56**, 437 (1929).
- Lewis, G. E. dissertation, Yale Univ. (1937).
- Dehm, R. *et al. Abh. Bayer. Akad. Wiss.* **90**, 5 (1958).
- Dehm, R. *Abh. Bayer. Akad. Wiss.* **114**, 5 (1963).
- Heissig, K. *Abh. Bayer. Akad. Wiss.* **152**, 5 (1972).
- Hoffstetter, R. *Bull. Soc. Geol. France* 7 ser., **6**, 467 (1964).
- Modell, H. *Abh. Bayer. Akad. Wiss.* **135**, 5 (1969).
- Black, C. C. *Palaeontology* **15**, 238 (1972).
- Fahlsbuch, V. *Neust. Stratigr.* **5**, 160 (1976).
- Gabounia, L. K. *Acad. Sci. Rep. Soc. Georg. Inst. Palaeobiol.* **136** (1973).
- Berggren, W. A. & Van Couvering, J. *Palaeo. Palaeo. Palaeo.* **16**, 1 (1974).
- Hussain, S. T. *Abh. Bayer. Akad. Wiss.* **147**, 5 (1971).
- Van Couvering, J. & Miller, J. A. *Nature* **230**, 559 (1971).
- Sickenberg, O. *Geol. Jb.* **B15**, 1 (1975).
- Pickford, M. H. L. *Nature* **256**, 279 (1975).
- Simons, E. L., Pilbeam, D. & Boyer, S. J. *Nature* **229**, 408 (1971).
- Prasad, K. N. *J. Geol. Soc. India* **3**, 86 (1962).
- Prasad, K. N. *Palaeontology* **7**, 1 (1964).
- Jacobs, L. L. *Paleobios* **25** (1977).

## New hominoid primates from the Siwaliks of Pakistan and their bearing on hominoid evolution

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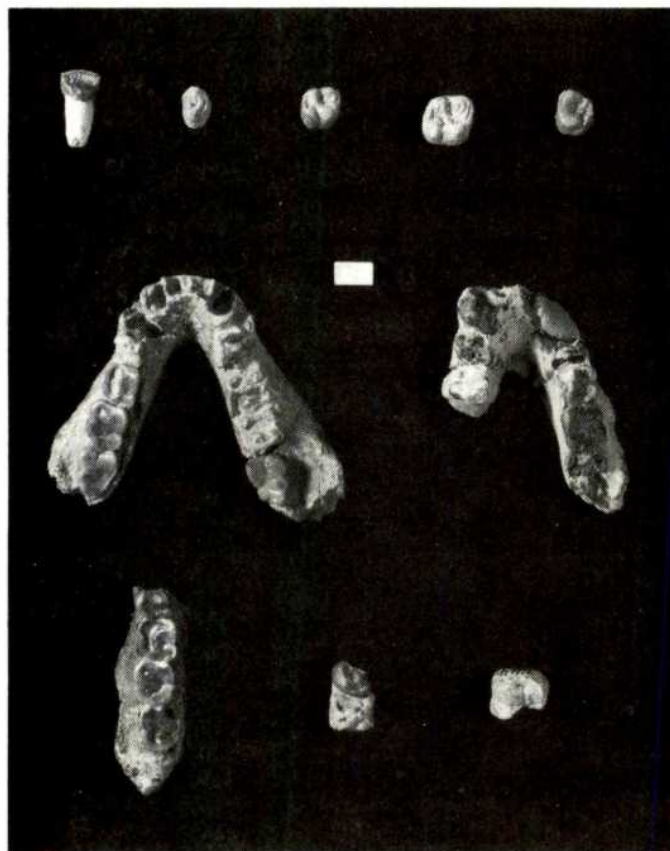
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*Siwalik deposits in the Punjab have yielded a rich collection of hominoid primate remains. Together with other recent finds they indicate the need for some changes in hominoid classification.*

SINCE 1973 a team from the Geological Survey of Pakistan (GSP) and the Peabody Museum has collected fossils in the Potwar

Plateau, Punjab Province, Pakistan (72°30' E, 33°00' N) as part of a joint research project aimed at a better understanding of the geological, floral and faunal history of South Asia. Neogene rocks of the Siwalik Group are widely exposed in India and Pakistan as part of the South Asian alpine system. The Soan synclinorium in the Potwar Plateau is the area in which all but one of the type localities for Siwalik formations and South Asian Land Mammal





**Fig. 1** Top row left to right: GSP 9903, 9906, 5019, 6206, 8702. Middle row: GSP 4622/4857, 9563/9902. Bottom row: GSP 6153, 7619, 7144.

Ages are located (Figs 1 and 2 of preceding article). The lithostratigraphy and biostratigraphy of Siwalik deposits in the Plateau have been discussed in the preceding article<sup>1</sup>.

More than 13,000 catalogued fossil specimens representing a diverse vertebrate and invertebrate fauna have been collected from more than 300 localities; 18 localities have yielded 86 new hominoid primate specimens representing a minimum of 43 individuals (Tables 1–3). The hominoids are accurately located stratigraphically with well documented information concerning lithological context and faunal associations. They approximately double the previously known hominoid collections<sup>2</sup>. In addition, new stratigraphic information has facilitated further interpretation of earlier collections. Of particular interest are four localities (Table 2) in which remains of between five and nine hominoid individuals have been found together.

Detailed descriptions of the geology, revisions of major vertebrate and invertebrate groups, and more extensive descriptions and analyses of the hominoids will be published elsewhere as studies are completed. What follows is a preliminary announcement of the primate finds.

### Geology and palaeoecology

Most of the new hominoid sites are along the northern rim of the Soan synclinorium between Dhok Mila and Kaulial. One, 311, is at Sethi Nagri, another, 38, south of Chinji; both are along the southern margin of the synclinorium. All the northern sites, with two exceptions (251 and 261), are situated stratigraphically in the upper third of the Nagri Formation; localities 251 and 261 are somewhat lower in the section. Locality 311 at Sethi Nagri is probably situated somewhat below the level of the bulk of the northern sites. Locality 38 is in the Chinji Formation.

Approximate ages can be assigned to the localities on the basis of faunal comparisons to dated sequences elsewhere in the Old World, and these are apparently supported by palaeomagnetic surveys which will be published soon. Locality 38 may be about 12 Myr old; 251 and 261 about 9.5–10 Myr old; and the rest of the

sites some 9.0 Myr old.

Abundant vertebrate and invertebrate faunal remains have been recovered from the Potwar Plateau both by us and by previous expeditions, and the biostratigraphic sequence is becoming clear. The upper Nagri levels are particularly well sampled, and new analyses of major taxonomic groups (rodents, carnivores, suids, bovids and equids) suggest close similarities to European and West Asian sites of late Vallesian and early Turolian age<sup>3</sup>.

Earlier studies of Siwalik lithology<sup>4</sup>, faunas<sup>5–7</sup> and floras<sup>8,9</sup> generated hypotheses about past habitats. These studies were large scale, involving the analysis of faunas from many localities and broad stratigraphic ranges, or used lithological or floral samples which could not be tied into firm stratigraphic or palaeogeographic frameworks. Only recently have more adequate studies started<sup>10</sup>. Our programme of detailed lithostratigraphical, magnetostratigraphical, sedimentological, taphonomical, faunal and floral analyses is aimed at a better understanding of Siwalik palaeogeography and past plant and animal associations.

Many primate localities are in pellet rock lithologies usually associated with minor grey-green sandstones within a red-bed sequence. In localities where several individuals are preserved, primates are often a relatively abundant component of the fauna. So far collections have been made in a taphonomically adequate way from few sites, nor have faunal analyses involving minimum numbers of individuals been completed. Possible faunal differences between primate and non-primate localities are being investigated. Preliminary faunal analyses from our 'best-collected' localities suggest that the overall mammal community lacked a diversity of large mammals and had low numbers of grazing species, herbivores above 100 kg and arboreal species. By comparison with recent and Pleistocene faunas associated with riverine environments, this suggests woodland or bush habitats with open patches of grassland rather than extensive forests. The sedimentary features and lithologies of the upper Nagri indicate a braided fluvial regime of low sinuosity rather than a meandering one, and a rapidly aggrading multiple-channelled river would

**Fig. 2** Top row, left to right: GSP 11708, 11704, 9977/01/05/9564. Bottom row: 11536/7, 13163, 4230, 9977/01/05/9564.

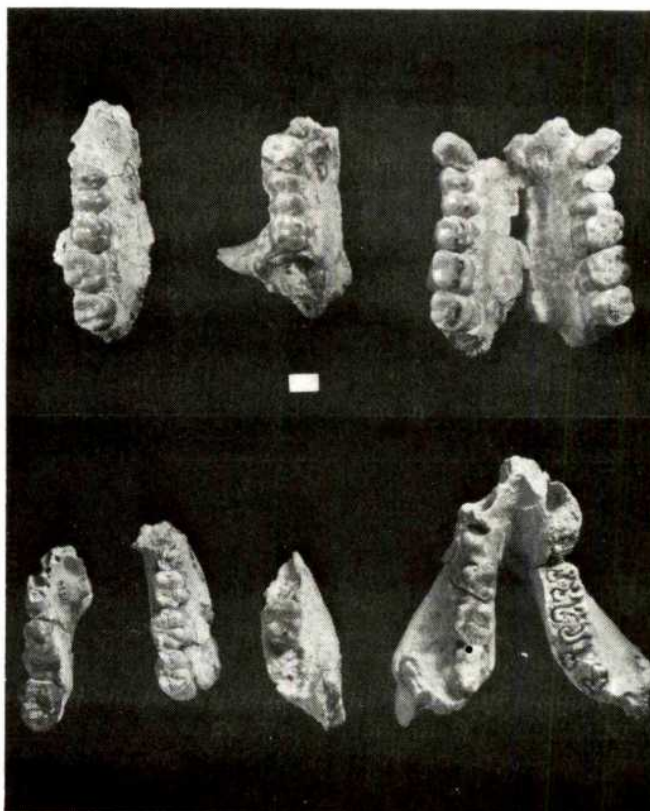


Table 1 Hominoid specimens collected from the Potwar Plateau, Punjab

Area	Locality	GSP no.	Specimen	Area	Locality	GSP no.	Specimen
Sethi Nagri	311	11536/7	Infant left mandibular corpus, dP <sub>3</sub> , dP <sub>4</sub> , associated M <sub>2</sub>			13166	Left P <sup>4</sup>
		10493	Left C			12647	Right M <sup>1</sup>
		11534	Left P <sup>4</sup> fragment			9972	Right M <sup>2</sup>
		11999	Right M <sup>1</sup>			9896	Left M <sup>2</sup>
		13162	Right M <sup>1</sup> fragment			9969	Right M <sup>2</sup>
		12000	Right M <sup>1</sup> fragment			9895	Left M <sup>3</sup>
		9986	Right M <sup>1</sup>			9900	Right M <sup>3</sup>
		10500	Left M <sup>2</sup> fragment			12709	Infant mandible, right C, dP <sub>3</sub>
		11533	Right M <sup>2</sup> fragment			9563/9902	Mandible, left P <sub>3</sub> , M <sub>1</sub> , right M <sub>2</sub> , M <sub>3</sub>
		7144	Left M <sub>2</sub> fragment			13165	Right mandibular fragment, M <sub>1</sub> - M <sub>3</sub>
		11998	Right M <sub>2</sub>			9565	Left C fragment
		13164	Central I			9899	Right M <sub>3</sub>
		12648	Central I			12654	Femoral fragments
		6663	Distal humeral epiphyseal fragment			9894	Femoral head fragment
		6664	Distal pollical phalangeal fragment			13168	Distal phalangeal fragment
		6666	Distal phalangeal fragment	Khaur	317	11708	Right maxilla, P <sup>3</sup> - M <sup>3</sup>
		6454	Intermediate cuneiform			11786	Right maxillary fragment, P <sup>3</sup> - M <sup>2</sup>
		12271	Distal humeral fragment			11704	Right maxillary fragment, I <sup>2</sup> root - M <sup>1</sup>
		8928	Right I <sup>1</sup>			7618	Right M <sup>2</sup>
		8925	Right C			11706	Right mandibular fragment
		5019	Right M <sup>1</sup>			11707/85	Mandible, left M <sub>2</sub>
		5018	Left M <sup>2</sup> fragment			7619	Left mandibular fragment, P <sub>3</sub>
		5067	Left M <sup>3</sup>			9930	Left M <sub>2</sub>
		8702	Left M <sup>3</sup>			7611	Radial diaphysis
		4622/4857	Mandible, left M <sub>1</sub> - M <sub>3</sub> , right M <sub>3</sub>			11867	Femoral head and neck
		4230	Right mandibular fragment with M <sub>2</sub>			3293	Left I <sup>1</sup>
		5464	Right I <sub>1</sub>	Khaur	137	12568	Left C
		8679	Right C			5001	Right M <sub>2</sub>
		5712	Left P <sub>3</sub> fragment			5260	Right M <sub>1</sub>
		5020	Left P <sub>3</sub>			6758	Left M <sup>3</sup>
		4635	Right M <sub>2</sub>			6759	Right M <sub>3</sub>
		4735	Right M <sub>3</sub>			6153	Left mandibular fragment, P <sub>4</sub> - M <sub>3</sub>
		8926	Right M <sub>3</sub>			7308	Right M <sup>1</sup> fragment
		8927	Left M <sub>3</sub>			6178	Femoral head fragment
		4664	Left distal calcaneal fragment			6160	Right mandibular fragment, P <sub>3</sub> - M <sub>3</sub>
Khaur	182	9977/01/05/	Maxilla, left and right C, M <sup>3</sup>			6206	Right M <sup>2</sup>
		9564	right I <sup>2</sup> ; mandible, right C, left P <sub>3</sub> - M <sub>3</sub> ; right condyle; Cranial and facial fragments			13171	Left I <sup>1</sup>
		9897	Left maxillary fragment			6999	Right I <sup>1</sup>
		9903	Right I <sup>1</sup>			8836	Right M <sup>2</sup>
		9898	Left I <sup>1</sup>			9987	Right P <sup>4</sup>
		13167	Left C			11597	Left C
		9906	Left P <sup>4</sup>			1023	Left C fragment
						10785	Talar fragment
						11003	Left C
				Chinji	38	780	Left C fragment

probably have been characterised by a mosaic of more and less open habitats rather than extensive riparian forest.

There have been too few palynological studies of Siwalik age sediments<sup>8-10</sup> to indicate clearly either the range of habitats at a particular time or habitat changes through time. Available evidence suggests a change during the deposition of the Chinji and Nagri Formations from mainly subtropical, forest habitats to more open, less low-lying habitats. Schaller<sup>11</sup> has summarised views on the modern vegetation of the area: evergreen and semi-evergreen forests have quite restricted distributions, most habitats being deciduous forest or thorn forest. Although South Asian Neogene climates may have been warmer and with more precipitation than Recent climates, it is possible that evergreen forests (including gallery forests) were never very widespread during the time of deposition of Siwalik group rocks. As noted, such limited information as we have suggests non-evergreen forest contexts for at least some of the major primate localities.

## Primates

There is an extensive primary literature on Siwalik primates, and they have been mentioned and discussed many times<sup>2</sup>. Approximately 90 hominoid specimens representing more than 60 in-

Table 2 Tentative listing of minimum numbers of hominoid individuals

Locality	No. of specimens	Minimum nos individuals			
		G	R	Si	Total
182	17	-	4	2	6
260	21	-	4	5	9
311	19	1	5	5	6
317	10	-	2	3	5
137	1	-	-	1	1
191	1	-	-	1	1
207	1	-	-	1	1
211	1	-	-	1	1
221	2	-	1	-	1
224	3	-	2	-	2
226	1	-	2	-	1
227	2	-	2	-	1
230	1	-	-	1	1
251	1	-	1	-	1
261	1	-	-	1	1
309	2	-	-	2	2
310	1	-	2	-	1
314	1	-	-	1	1
38	1	-	-	1	1
19	86	1	17	25	43

G, *Gigantopithecus*; R, *Ramapithecus*; Si, *Sivapithecus indicus*.



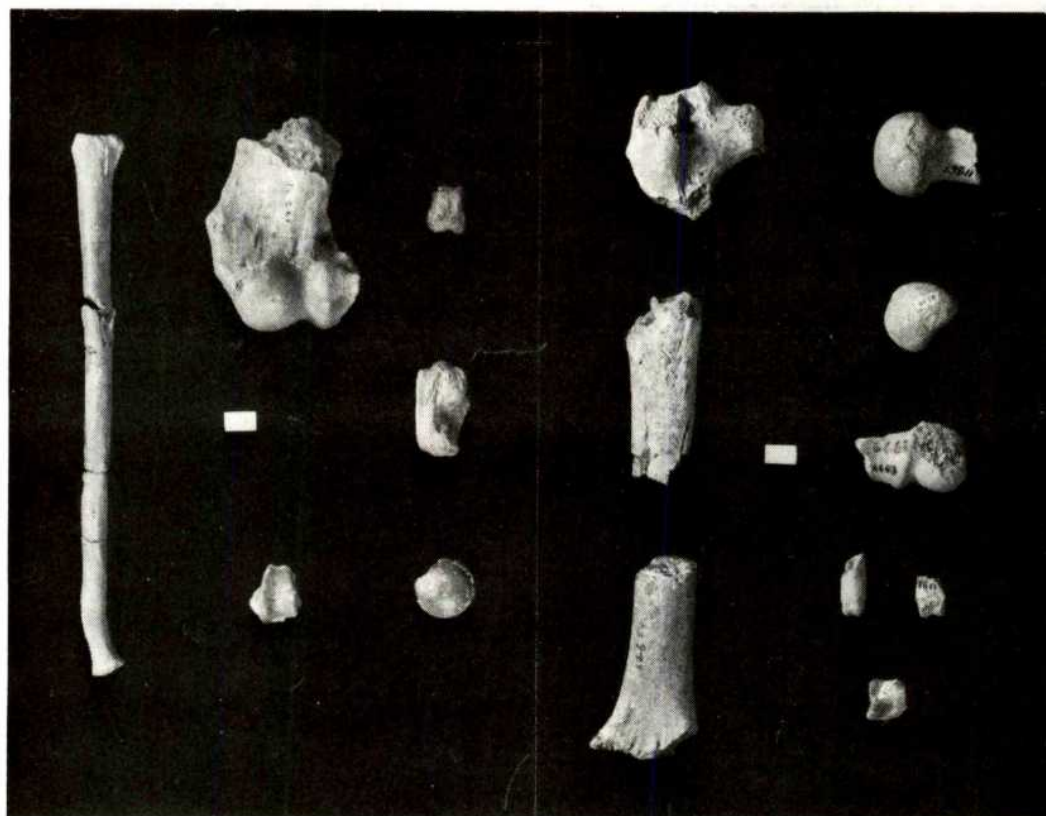


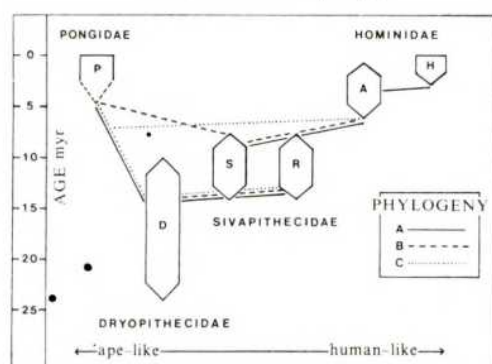
Fig. 3 Left to right, first column: GSP 7611; second column: top, GSP 12271, bottom, GSP 10785; third column: top, GSP 6664, middle, GSP 4664, bottom, GSP 9894; fourth column, GSP 12654; fifth column, top to bottom, GSP 11867, 6178, 6663, 6666 and 13168, 6454.

dividuals are known from previous collections. The Potwar Plateau yielded some isolated teeth and a few jaws, the bulk from the Chinji Formation at Chinji; several specimens came from around Hasnot, and a few individual teeth were known from other localities. Ramnagar in Kashmir was the source of a few hominoids, but the best collections came from Haritalyangar in India.

At least four hominoid species are represented in the Siwaliks, thought previously to range in age between about 14 Myr and 6 Myr. The four species are *Ramapithecus punjabicus*, *Sivapithecus sivalensis*, *S. indicus* and *Gigantopithecus bilaspurensis*. Until recently species of *Sivapithecus* have been classified in *Dryopithecus*, but it now seems preferable to separate them generically from *D. fontani* and other dryopithecines.

In several recent reviews of Miocene Hominoidea these Siwalik species and others from elsewhere in the Old World have been placed in either the Hominidae or Pongidae. *Ramapithecus* has often been included in Hominidae, while species of the other genera have usually been considered pongids<sup>1,2</sup>. This arrangement has not been accepted universally.

Fig. 4 Tentative stratigraphic-morphological distribution of large hominoid groups with possible phylogenies. P, Pongidae; D, Dryopithecidae; S, Sivapithecinae; R, Ramapithecinae; A, Australopithecinae; H, Homininae. (Sivapithecidae should be Ramapithecidae; note added in proof.)



The new specimens from Pakistan discussed here (Tables 1–3), together with much new material from other Old World localities, facilitate a different view of earlier Neogene hominoid classification and evolution.

The new hominoids are discussed here taxonomically. Assignment of some individuals to species is difficult or impossible, and the scheme outlined is provisional. More comprehensive descriptions and discussions are being prepared. Measurements of the more complete specimens are given in Tables 4 and 5.

At least one species of *Ramapithecus*, *R. punjabicus* (Fig. 1), is represented in Siwalik rocks; the genus is found in the Chinji and Nagri Formations, and possibly in rocks equivalent to the Dhok Pathan Formation. The probable age range is 13–8.5 Myr.

The new material from the Potwar Plateau helps considerably in understanding previous finds as well as adding significant new information. Particularly fine specimens are the adult mandibles GSP 4622/4857 from locality 182 and GSP 9562/9902 from

Table 3 Distribution of hominoid parts

Part preserved	Specimens
Cranial	
Associated maxillae and mandibles	1
Adult maxillae	4
Adult mandibles	9
Infant mandibles	2
Isolated teeth	
I <sup>1</sup>	6
C	8
P <sup>4</sup>	4
M <sup>1</sup>	6
M <sup>2</sup>	11
M <sup>3</sup>	6
I <sup>1</sup>	3
C	2
P <sup>3</sup>	1
P <sup>4</sup>	1
M <sup>1</sup>	1
M <sup>2</sup>	3
M <sup>3</sup>	5
Postcranial	13



**Table 4** Tooth measurements on hominoid maxillae

		9977/01		11704	11736	11708
		L	R	R	R	R
I <sup>2</sup>	md	—	5.5			
	bl	—	7.5			
C	max	14.2	13.4	14.6		
	tr	10.8	10.8	10.7		
P <sup>3</sup>	md	9.3	9.3	9.2	9.2	9.5
	bl	11.8	14.3	11.2	—	11.6
P <sup>4</sup>	md	8.7	7.5	7.7	8.5	8.3
	bl	12.4	12.4	11.7	12.2	12.0
M <sup>1</sup>	md	11.5	12.1	11.1	12.0	12.4
	bl	13.3	13.3	12.8	—	13.1
M <sup>2</sup>	md	13.5	13.6		12.5	13.7
	bl	14.6	14.2		13.4	14.0
M <sup>3</sup>	md	12.7	12.7			12.5
	bl	13.7	13.5			13.8

locality 260, and an infant mandible, GSP 12709, also from locality 260. These specimens show that the incisor region was very narrow in *Ramapithecus*, canines small, anterior premolars and canines closely packed together, and postcanine tooth rows and mandibular corpora posteriorly divergent. Most teeth in the upper and lower dentitions are now known. Certain features are worth noting: P<sub>3</sub> has a small but distinct lingual cusp, and its long axis is oriented at some 45° to the mesio-distal line of the tooth row. Unworn cheek teeth resemble those of *Australopithecus* and *Homo* quite strongly, particularly the maxillary molars. Occlusal surfaces are constricted and there is marked buccal (mandibular teeth) and lingual (maxillary) flare in unworn specimens (compare ref. 13). Occlusal surfaces broaden with wear and can be almost flat and still show no dentine. This is because occlusal enamel is very thick (between 2.5 and 3.0 mm on mandibular buccal cusps), as shown in a few broken specimens (GSP 8926) and by comparison of unworn and worn homologues of similar size. Mandibular rami are relatively robust; symphyses have marked superior and inferior transverse tori.

*Gigantopithecus bilaspurensis* (Fig. 1) is based on a complete mandible from the Haritalyangar area. It is likely to have an age of around 8.5 Myr<sup>1,2,10</sup>, and is not significantly younger than the other hominoid primates from that area.

One partial molar from locality 311, GSP 7144, is tentatively assigned to *Gigantopithecus*, as is a previously described molar, GSI D175 from Alipur near Hasnot<sup>14</sup>. Both are probably 9.0–10 Myr old. Occlusal morphology is rather similar to that of

*Ramapithecus*, and occlusal surface enamel is thick (about 3.5 mm on the mandibular buccal cusp of GSP 7144).

Specimens of *Sivapithecus indicus* (Fig. 2) are known from deposits ranging between about 13 and 8 Myr. Several relatively complete new specimens (especially GSP 9977/01/05/9564 from locality 260) facilitate mandibular and lower facial reconstructions. Tooth rows are subparallel with broad incisor regions and postcanine tooth rows that are markedly concave buccally.

Occlusal morphology in *S. indicus* resembles the other genera, although molars are somewhat broader and there are other minor differences. Occlusal surfaces are constricted on cheek teeth with marked buccal and lingual flare; enamel is thick (about 3 mm on mandibular buccal cusps).

Canines are projecting and moderately dimorphic and exhibit mesial, distal and apical wear; P<sub>3</sub>s are closely approximated to the canines, their long axes rotated to lie about 45° to the mesio-distal line of the tooth row. Mandibular rami are deep; the symphysis is long with a prominent inferior transverse torus and a relatively small superior transverse torus.

*S. sivalensis* is the most enigmatic of the Siwalik hominoids and it is not absolutely clear that it exists as a separate species.

Parts of 13 hominoid postcranial bones (Fig. 3) have been collected so far and fit into three size groups. The largest specimen is a partial distal right humerus, GSP 12271, with the lateral supracondylar ridge, lateral epicondyle, capitulum and radial fossa preserved entire, and the coronoid and olecranon fossae and trochlear surface preserved in part. In its size and morphological features this specimen is similar to the distal humerus of adult female gorillas. Eight specimens are close in size to the corresponding bones of adult pygmy chimpanzees. This group includes a partial distal right humerus, GSP 6663, with parts of the capitular and trochlear surfaces preserved. Hindlimb specimens include a femoral head, GSP 6178, a femoral head plus part of the neck, GSP 11867, the proximal, mid-shaft, and distal non-articular parts of a right femur, GSP 12654, a left intermediate cuneiform, GSP 6454, and the distal end of a proximal hallux phalanx, GSP 6664. These specimens, together with two distal ends of phalangeal bones, GSP 6666 and GSP 13168, show some morphological similarities to extant hominoid species, although these are not as marked as in the case of the large specimen. Three fossils are smaller. One of them, a partial left radius, GSP 7611, is clearly juvenile. A partial right talus, GSP 10785, which includes most of the trochlear surface, the lateral process, and most of the posterior calcaneal articular surface, and a partial femoral head, GSP 9894, come from approximately adult macaque-sized animals. It is not certain that they are from adults. A partial calcaneus,

**Table 5** Measurements of hominoid mandibles

		9564/9905	13165	4622/4857	4230	9563/9902	6153	6160
C	max	12.3						
	tr	10.1(R)						
P <sub>3</sub>	max					11.2		11.0
	tr					7.0		7.0
P <sub>4</sub>	md	* 9.4(L)					8.1	8.5
	bl	—				—	9.5	9.8
M <sub>1</sub>	md	*13.0	13.0	11.5		*10.5	10.3	11.1
	bl	*12.0(L)	*10.9	9.5		* 9.5	9.8	—
M <sub>2</sub>	md	14.5	14.0	12.7	14.7	12.7	12.4	12.7
	bl	12.7(L)	11.7	10.5	12.7	10.7	10.9	10.7
M <sub>3</sub>	md	*15.5(L)	*14.5	12.9		—	12.3	13.7
	bl	—	—	10.5	—	—	10.8	—
Breadth at C		*35		22.5		*25		
Breadth at M <sub>2</sub>		*47		48.0		*40		
Symphysis								
Depth		52.5		*30.0		*34		
Thickness		20.0		15.0		*16		
At P <sub>4</sub>								
Depth		43.5	34.0	30.5		*28		
Thickness		15.5	15.0	13.0		*14		
At M <sub>3</sub>								
Depth		42.5		31.0	30.5			
Thickness		24.0		20.0	23.5			

\*Estimated values.

GSP 4664, shows a number of equivocal morphological features and is not included in any of the three groups.

None of these specimens was found in direct association with cranial or dental material. Because only three different sized primate species are definitely represented by cranial and dental specimens, however, the largest postcranial specimen is provisionally assigned to *Gigantopithecus* cf. *bilaspurensis*, the intermediate sized group to *Sivapithecus indicus*, and the smaller specimens to *Ramapithecus punjabicus* (remembering that some of these specimens may represent juveniles of the middle size group). It is significant that the *Gigantopithecus* humeral fragment and many of the *Sivapithecus* specimens come from locality 311; dental remains from that locality include our only *Gigantopithecus* specimen and five *Sivapithecus indicus* (Table 2).

If the postcranial remains are correctly subdivided and if they are associated with the three size groups based on gnathic and dental remains, some interesting conclusions can be drawn.

First, all hominoid species in the Siwaliks apparently have cheek teeth with very thick occlusal enamel. Second, all are truly megadont, in that cheek teeth are very large relative to body size. In these two features, the Siwalik hominoids resemble Plio-Pleistocene hominids and differ from pongines and the Miocene hominoids now assigned to Dryopithecinae (*sensu strictu*, see below). Third, these species share a basically similar occlusal morphology.

The Siwalik hominoids subdivide into two groups, mainly on the basis of anterior tooth size. *S. indicus* has relatively large incisors and, like *S. sivalensis*, large dimorphic canines; *Ramapithecus* and *Gigantopithecus* species have relatively small incisors, (probably) non-projecting and (probably) moderately dimorphic canines.

## Other hominoid material

New ideas and new hominoid fossil remains recovered during the past decade suggest that earlier, simpler schemes of hominoid evolution need to be modified. These changes can be summarised briefly as follows.

(1) Important dental differences between later hominids and living pongids lie less in arcade shape and incisor size as often stated in the past, but in relative tooth size and occlusal enamel thickness. Living apes have U-shaped dental arcades, living humans parabolic ones. Such arcade shapes are rarely found in other Neogene hominoids, the predominant form being some variant of a V-shape. Incisor size also seems to have been rather variable at all phases of hominoid evolution. More important, the apes, like almost all other non-human higher primates, have relatively small cheek teeth with thin enamel, perhaps an adaptation to predominantly browsing diets. Pliocene and earlier Pleistocene hominids in contrast have large cheek teeth with thick enamel (*Homo sapiens* has evolved small cheek teeth relatively recently). Apes have large tusk-like sexually dimorphic canines; hominids have small, somewhat incisiform canines exhibiting considerably less sexual dimorphism.

(2) New discoveries in east Africa, and new analyses of earlier finds in Africa and Europe, suggest that early apes were considerably more diverse than previously believed<sup>15</sup>. Classifying them in only one genus, *Dryopithecus*, obscures this diversity. Thus at least three genera (*Proconsul*, *Rangwapithecus* and *Limnopithecus*) should probably be recognised in east Africa during the early and middle Miocene, separated from *Dryopithecus*, at least two species of which are found in middle Miocene deposits in Europe. These species can all be placed conveniently in the Dryopithecinae (or Dryopithecidae). All have dentitions basically like those of the living African pongids, with thin enamel and, if postcranial remains are correctly allocated, relatively small cheek teeth. Canine-premolar complexes are like those of modern apes.

Postcranial material of this group has proved difficult to interpret in a framework heavily dependent on comparisons with modern primate groups<sup>16</sup>. The fossil species were probably arboreal, and seem to be qualitatively different from living apes, being more 'monkey-like' in certain ways; they are probably best

viewed as truly primitive relative to modern hominoids.

Where palaeoecological contexts can be inferred plausibly, dryopithecine species seem to have been associated with predominantly forest floras and faunas<sup>15</sup>. Adaptively this rather diverse group was probably more like living ceboids or cercopithecoids than the low-diversity modern hominoids.

(3) Besides dryopithecines, other kinds of hominoids are present in the Old World middle Miocene. New material from Hungary<sup>17</sup>, Greece<sup>18</sup>, Turkey<sup>19</sup>, Kenya<sup>20</sup> and China together with the specimens described here from Pakistan as well as earlier fossils from Europe and Africa show that thick-enamelled and (at least for those with postcranial remains) megadont hominoid species were widely distributed between 14 and 8 Myr ago, probably in predominantly non-forested habitats. During this time cercopithecoid monkeys seem to have been absent from Eurasia, and not particularly diverse in Africa<sup>21</sup>.

This thick-enamelled middle Miocene cluster of species can be divided into two groups. One, consisting of species variously described as *Sivapithecus*, *Bodvapiithecus*, *Ankarapithecus* and *Ouranopithecus* contains forms that are rather more ape-like, with large and sexually dimorphic canines. More than one generic name is probably necessary to reflect adequately the diversity of this group, which would be termed Sivapithecinae (or Sivapithecini). The other group would contain species described as *Gigantopithecus*, *Ramapithecus* and *Rudapithecus* (at least), forms with canines smaller and less dimorphic than those of pongines, dryopithecines or *Sivapithecus*-group species, although perhaps more so than australopithecines. Canine-premolar complexes resemble those of 'primitive' australopithecines<sup>22</sup>. This cluster would be termed Ramapithecinae (or Ramapithecini).

(4) Recent discoveries suggest very strongly that the story of the Plio-Pleistocene hominids was more complex than previously thought. Between about 3.75 and 1 Myr ago several hominid lineages seem to have coexisted, certainly in Africa and possibly in Asia too<sup>23</sup>. These species have been classified in both *Australopithecus* and *Homo*, but are characterised by certain shared features: reduced canines exhibiting moderate size dimorphism, enlarged and thick-enamelled cheek teeth, relatively enlarged brains (compared with dryopithecines and pongines at least), and a postcranial skeleton showing numerous adaptations to habitual bipedalism. At least one of these species, by at least 2 and perhaps 2.5–3 Myr ago, made stone tools.

It has been increasingly realised that these so-called Plio-Pleistocene hominids are not to be regarded merely as 'diminutive humans' but as creatures, although recognisably hominid, qualitatively different from middle and late Pleistocene hominids. They are a diverse group of truly primitive species.

The oldest specimens with teeth similar to those of *Australopithecus* or early *Homo* species (and distinguishable from those of the *Sivapithecus* or *Ramapithecus* groups) come from Lukeino and Lothagam in Kenya and are between 5 and 7 Myr old<sup>24,25</sup>.

The period after about 7 Myr ago is one during which australopithecines and early hominines diversified; *Sivapithecus*-group and *Ramapithecus*-group species are so far unknown. In both Africa and Eurasia cercopithecoid monkeys, especially the more open-country types, become abundant for the first time.

## Synthesis

Current knowledge of hominoid evolution suggests a tentative taxonomic and phylogenetic scheme that reflects the important advances of the past decade. These are summarised here by D.P.

First, Neogene hominoids were, during most of their evolution, a relatively diverse superfamily. Second, extinct hominoids were not identical with, nor, in some cases, particularly similar to living hominoids, and to interpret extinct hominoids as though they were very 'modern' is potentially misleading. Third, it is very difficult to draw exact ancestor-descendant relationships given the complexity of the picture at any given time, the differences between descendants and available ancestral candidates, and the still substantial gaps in the fossil record. Rather, each radiation should be studied for its own sake in order to understand it as an adapted and successful group. Fourth, clearly the two taxonomic

categories into which living large hominoids are normally subdivided, Pongidae and Hominidae, cannot be imposed on earlier Neogene species without suppressing important information and obscuring evolutionary concepts.

A minimum of six clusters of species is needed to describe the diversity of Neogene large hominoids, and these can be classified as tribes or subfamilies.

(1) Ponginae: the living great apes. (2) Dryopithecinae: species from Africa and Europe, ranging in age from about 23 to 9 Myr, sharing dental features with pongines but differing in many postcranial characters; (3) Sivapithecinae or Sivapithecini: species from Eurasia and Africa, ranging in age from around 15 to 8 Myr, sharing dental features with both pongines and dryopithecines on the one hand and with australopithecines and early hominines on the other; (4) Ramapithecinae or Ramapithecini: Eurasian and African species resembling sivapithecines and australopithecines about equally in dental features; (5) Australopithecinae: species from Africa and possibly Asia ranging in age from at least 3.75 (and perhaps as much as 7) to 1 Myr; (6) Homininae: essentially similar to Australopithecinae in dental, cranial and postcranial morphology.

Figure 4 shows these groups distributed along temporal and qualitative morphological axes; the latter axis is shown as unidimensional although it is, of course, multidimensional. Three alternative phylogenies are indicated on the diagram in order of decreasing probability (in my opinion). Although definite relationships between the middle Miocene and Plio-Pleistocene hominoids are unclear because of gaps in the hominid record between 8 and 4 Myr and the pongid record after 9 Myr, I believe that the major groups as defined here represent at least some of the grades through which modern hominoids evolved. Phylogeny A is the most probable; I regard phylogenies B and C as less probable, although plausible.

I suggest the following classification of large Hominoidea, a compromise of 'vertical' and 'horizontal' philosophies<sup>26</sup>. (1) Pongidae; (2) Dryopithecidae: including Dryopithecinae and older, more primitive hominoids; (3) Ramapithecidae: Sivapithecinae (or Sivapithecini) and Ramapithecinae (or Ramapithecini);

(4) Hominidae: Australopithecinae and Homininae.

The classification is flexible in that it is compatible with any of the three phylogenies suggested in Fig. 4. Whether or not the four groups are classified as families, subfamilies or tribes, they should be coordinate. As the fossil record improves I expect that certain (perhaps known) members of Ramapithecidae and Hominidae will prove more conclusively to be ancestors and descendants (that is phylogeny A in Fig. 4 becomes more probable), in which case some workers might wish to include those (or all) ramapithecids in Hominidae. Finally, two important points should be re-emphasised. A rigidly dichotomous classification of Neogene large hominoids is not only inappropriate but potentially misleading. And the living hominoids are rather aberrant forms when the total array of Neogene large hominoids is considered.

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1. Pilbeam, D. *et al.* *Nature* **270**, 684–689 (1977).
2. Pilbeam, D. *Les Plus Anciens Hominidés* (eds Tobias, P. V. and Coppens, Y.) 39–59 (Centre National de la Recherche Scientifique, Paris, 1976).
3. Berggren, W. A. & Van Couvering, J. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **16**, no. 1/2 (1974).
4. Krynine, P. *Am. J. Sci.* **34**, 422–446 (1937).
5. Lewis, G. E. *Am. J. Sci.* **33**, 191–204 (1937).
6. Tattersall, I. *Nature* **221**, 451–452 (1969); **224**, 821–822 (1969).
7. Prasad, K. N. *Nature* **232**, 413–414 (1971).
8. Banerjee, D. *Rev. Palaeobotan. Palynol.* **6**, 171–176 (1968).
9. Nandi, B. *Himalayan Geol.* **5**, 411–424 (1975).
10. Johnson, G. D. *Geol. Rundschau*, **66**, 192–216 (1977).
11. Schaller, G. *The Deer and the Tiger* (Chicago University Press, 1967).
12. Simons, E. L. *J. Hum. Evol.* **5**, 511–528 (1976).
13. Simons, E. L. *Proc. natn. Acad. Sci. U.S.A.* **51**, 528–535 (1964).
14. Pilgrim, G. E. *Rec. Geol. Surv. India* **45**, 1–74 (1915).
15. Andrews, P. & Van Couvering, J. *Approaches to Primate Paleobiology* (ed. Szalay, F.), 62–103 (Karger, Basel, 1975).
16. McHenry, H. & Corruccini, R. *Folia Primat.* **23**, 227–244 (1975).
17. Kretzoi, M. *Nature* **257**, 578–581 (1975).
18. de Bonis, L. & Melentis, J. C. *r. Lebd. Séanc. Acad. Sci.* **284**, 1393–1396 (1977).
19. Tekkaya, I. *Bull. Min. Res. Expl. Inst. Turkey* no. 83, 148–165 (1974).
20. Andrews, P. & Walker, A. in *Human Origins* (eds Isaac, G. & McCown, E.) 279–304 (Benjamin, New York, 1976).
21. Delson, E. *Approaches to Primate Paleobiology* (ed. Szalay, F.) 167–217 (Karger, Basel, 1975).
22. Johanson, D. & Taieb, M. *Nature* **260**, 293–297 (1976).
23. Leakey, R. & Walker, A. *Nature* **261**, 572–574 (1976).
24. Behrensmeier, A. *Earliest Man and Environments in the Lake Rudolf Basin* (eds Coppens, Y., Howell, F., Isaac, G. & Leakey, R.) 163–170 (Chicago University Press, 1976).
25. Pickford, M. *Nature* **256**, 279–284 (1975).
26. Simpson, G. G. *Principles of Animal Taxonomy* (Columbia University Press, New York, 1961).

# A mutation that impairs the ability of lipoprotein receptors to localise in coated pits on the cell surface of human fibroblasts

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*Low density lipoprotein is taken up by cultured human fibroblasts through an endocytic process that requires the binding of the lipoprotein to specific receptors located in coated pits on the cell surface. The coated pits are discrete segments of the plasma membrane that can undergo rapid invagination to form coated endocytic vesicles. In one form of the human genetic disorder familial hypercholesterolaemia, the responsible mutation produces altered lipoprotein receptors that lack the ability to become incorporated into coated pits. Instead, these mutant receptors are scattered at random over the entire plasma membrane. Because of their mislocation on the cell surface, the mutant lipoprotein receptors are unable to carry their bound lipoprotein into the cell. The occurrence of this 'receptor mislocation mutation' provides strong evidence for the role of the coated pit in the receptor-mediated uptake of lipoproteins. The data also have implications for the structure and assembly of plasma membranes in mammalian cells.*

COATED pits on the cell surface constitute specialised membrane regions that mediate the uptake of macromolecules through adsorptive endocytosis<sup>1–5</sup>. In cultured human fibroblasts, these coated pits appear in thin-section electron micrographs as short (0.5  $\mu$ m) segments of the plasma membrane where the membrane is indented, thickened, and coated on its cytoplasmic surface by a filamentous material that frequently assumes a spike-like configuration<sup>4,6,7</sup>. Using the freeze-fracture/deep-etching technique, the coated pits have also been visualised in two dimensions on the fibroblast surface where they appear as shallow, crater-like depressions that occupy about 2% of the surface area. In freeze-fracture preparations, the membrane of these coated pits can be distinguished from the membrane in noncoated regions by the presence of a higher concentration of intramembranous particles<sup>2,5</sup>.

## Coated pits as site of lipoprotein receptors

In human fibroblasts, coated pits of the plasma membrane have been shown by both conventional transmission electron microscopy<sup>4,6,7</sup> and freeze-etching techniques<sup>2,5</sup> to contain specific receptors for low density lipoprotein (LDL), the predominant



transport protein for cholesterol in human plasma. Through the use of LDL covalently coupled to ferritin, we recently demonstrated that 50–80% of LDL receptors are concentrated within the 2% of cell surface that constitutes the coated pits<sup>4,6,7</sup>. The LDL-ferritin bound to its receptor in coated pits is internalised within several minutes when the coated pits invaginate to form coated endocytic vesicles<sup>4</sup>. The coated vesicles containing LDL migrate through the cytoplasm until they fuse with lysosomes<sup>4</sup>. The lysosomal acid hydrolytic enzymes then cleave the cholesteryl esters and release the free cholesterol of the lipoprotein. The liberated sterol becomes available for metabolic utilisation by the cell (for review, see refs 8, 9).

On the basis of previous studies, we postulated that the rapid internalisation of receptor-bound LDL is strictly dependent on the localisation of LDL receptors in coated pits<sup>4</sup>. We also suggested that these coated pits may contain other cell surface receptors and that the invagination of these pits into the cell every few minutes would provide a mechanism by which a variety of receptor-bound molecules could be transported rapidly into the cell and delivered to lysosomes<sup>4</sup>. A corollary of this hypothesis is that if a receptor were not contained within such a coated pit region, then it would not be able to transport its bound ligand to cellular lysosomes with high efficiency.

### Role of $R^{b+,i^+}$ allele in internalisation of LDL

Recently, a human mutation that allows testing of the above hypothesis has been discovered. This mutation was observed in fibroblasts cultured from a 14-yr-old (J.D.) who has the clinical phenotype of homozygous familial hypercholesterolaemia (FH), a hereditary disorder characterised by a marked increase in the plasma level of LDL<sup>10</sup>. Unlike 30 other subjects with the homozygous form of FH whose fibroblasts exhibit a primary defect in the binding of LDL to the receptor<sup>11</sup>, J.D.'s cells bind normal amounts of <sup>125</sup>I-labelled LDL at the receptor site, yet the receptor-bound lipoprotein fails to be internalised and consequently it is not degraded within cellular lysosomes<sup>10</sup>. This uptake defect is specific for LDL since the J.D. cells show normal bulk-fluid endocytosis of <sup>14</sup>C-sucrose and <sup>125</sup>I-labelled gamma globulin<sup>10</sup> as well as normal receptor-mediated binding, internalisation, and lysosomal degradation of <sup>125</sup>I-labelled human epidermal growth factor, a molecule that is metabolised by human fibroblasts in a manner similar to LDL (ref. 12 and our unpublished observations).

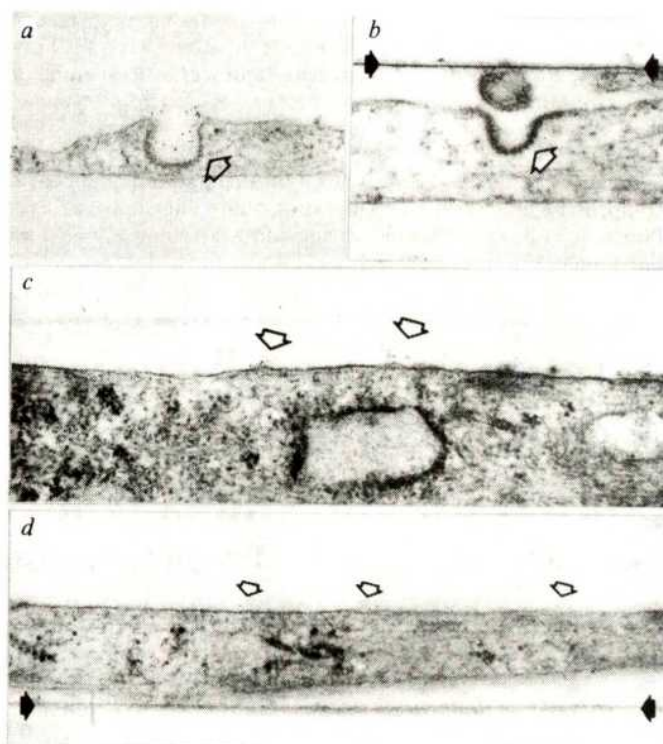
Biochemical studies of the fibroblasts of J.D.'s family members have disclosed that J.D. possesses two different mutant alleles at the LDL receptor locus<sup>13</sup>. From his mother, he has inherited the  $R^b$  allele that specifies a receptor molecule that is unable to bind <sup>125</sup>I-LDL normally and is thus biochemically silent. From his father, he has inherited the  $R^{b+,i^+}$  allele that specifies a receptor that can bind <sup>125</sup>I-LDL normally but cannot facilitate the internalisation of the receptor-bound lipoprotein. Because these two defects fail to complement each other in J.D. (genotype,  $R^b/R^{b+,i^+}$ ), we reasoned that they represent allelic mutations that disrupt different sites on the LDL receptor protein. The  $R^b$  mutation involves the site necessary to bind LDL, and the  $R^{b+,i^+}$  mutation involves a site necessary to couple the binding and internalisation processes<sup>13</sup>.

### $R^{b+,i^+}$ allele is a 'receptor mislocation mutation'

The discovery of the  $R^{b+,i^+}$  allele raises intriguing questions as to the relation between the mutant receptors specified by that allele and the coated pit of the plasma membrane. Accordingly, we have used LDL labelled with ferritin and <sup>125</sup>I to examine the localisation of LDL receptors in the J.D. cells at the electron microscopic and light microscopic levels, respectively. The results indicate that the  $R^{b+,i^+}$  mutation in the J.D. cells affects the LDL receptor in such a way that it cannot be incorporated into coated pits. The resultant mislocation of LDL receptors on the cell surface precludes the endocytosis of the receptor-bound lipoprotein.

Figure 1a shows a deeply invaginated coated pit of a normal fibroblast as it appears in thin sections of cells that have been incubated with ferritin-labelled LDL for 2 h at 4 °C, then fixed, and embedded while still in monolayer culture. Typically, most of the LDL-ferritin particles (open arrow) are clustered within the coated pit. In this experiment approximately 85% of the identifiable coated pits contained LDL-ferritin particles. In the J.D. fibroblasts (Fig. 1b), the coated pits were indistinguishable from the coated pits observed in normal cells (Fig. 1a and refs 4, 6, 7). In particular, their size, configuration, and distribution over both the top and bottom surfaces of the cell appeared the same in the J.D. and normal fibroblasts. In two experiments in which at least 2 mm of cell surface were examined per experiment, the number of coated pits per mm in the normal and J.D. cells incubated at 4 °C averaged 27 (25 and 29) and 19 (22 and 16), respectively. In two experiments in which the cells were incubated at 37 °C, the number of coated pits per millimetre of cell surface in the normal and J.D. cells averaged 13 (11 and 14) and 10 (10 and 10), respectively. The lower number of identifiable coated pits in normal cells at 37 °C as compared to 4 °C has been noted in previous studies and presumably is due to an altered morphology at the two temperatures<sup>4</sup>.

Despite a normal appearance and nearly normal number of coated pits in the J.D. cells, virtually no LDL-ferritin was bound in these specialised membrane regions (Fig. 1b). Rather, as shown in Fig. 1c and d, in the J.D. cells LDL-ferritin particles (open



**Fig. 1** Electron micrographs that show the localisation of LDL-ferritin on the cell surface of normal (a) and J.D.'s (b–d) fibroblasts. Monolayers of fibroblasts were prepared in 60 × 15 mm Petri dishes as previously described<sup>4</sup> and used for experiments in the late logarithmic phase of growth after a 48-h incubation in the presence of 5% (v/v) human lipoprotein-deficient serum<sup>23</sup>. On the day of the experiment (day 7 of cell growth), monolayers were cooled to 4 °C for 30 min. The medium was removed and replaced with 2 ml of ice-cold growth medium containing 5% human lipoprotein-deficient serum and LDL-ferritin at a concentration corresponding to 58 µg ml<sup>-1</sup> of LDL-protein. After incubation at 4 °C for 2 h, each monolayer was washed five times at 4 °C with ice-cold phosphate-buffered saline, fixed *in situ* with 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.3), embedded *in situ*, sectioned, and stained for electron microscopy<sup>4,6</sup>. The LDL-ferritin complex was prepared as previously described<sup>4,6</sup>. By negative staining electron microscopy, 2 to 3 ferritin particles were coupled to each LDL particle. The open arrows denote LDL-ferritin particles. The solid arrows denote the layer of serum proteins that coats the surface of the culture dish. 58,000 ×.



**Table 1** Quantitative analysis of binding of LDL-ferritin to coated pits of plasma membrane of normal and mutant fibroblasts

Cell strain	Genotype	Ferritin cores bound (no. per mm of cell surface)			% of bound ferritin cores associated with coated pits	<sup>125</sup> I-LDL bound (ng per mg protein)	
		In coated pits (a)	In non-coated regions (b)	Total (a + b)		Total	High affinity
Normal	+/+	186	195	381	49%	130	125
Normal	+/+	186	165	351	53%	121	116
J.D.	R <sup>b</sup> /R <sup>b+.</sup>	10	342	352	3%	99	93
Father of J.D.	+ / R <sup>b+.</sup>	112	444	556	20%	194	188

Cell monolayers were prepared as described in the legend to Fig. 1. On the day of the experiment (day 7 of cell growth), the fibroblast monolayers were cooled to 4 °C for 30 min. The medium was removed and replaced with 2 ml of ice-cold growth medium containing 5% lipoprotein-deficient serum and either LDL-ferritin (58 µg ml<sup>-1</sup> of LDL-protein) or <sup>125</sup>I-LDL (5 µg ml<sup>-1</sup> of LDL-protein, 212 c.p.m. per ng protein). The <sup>125</sup>I-LDL was added in the absence and presence of 300 µg protein per ml of unlabelled LDL. For the dishes that received LDL-ferritin, the monolayers were washed, fixed, embedded, and sectioned for electron microscopy as described previously<sup>4,6</sup>. Quantitation of the LDL-ferritin binding was performed on unstained sections as previously described<sup>6</sup> except that the sections were mounted directly from the diamond knife boat on to 100/400 mesh grids. Each value represents the average of duplicate monolayers. A total of about 2 mm of cell surface membrane was counted for each monolayer. For the dishes that received <sup>125</sup>I-LDL, the monolayers were washed six times at 4 °C with an albumin-containing buffer<sup>14</sup>, and the amounts of total and high affinity <sup>125</sup>I-LDL bound to the cell surface were determined as previously described<sup>14</sup>. The values for high affinity <sup>125</sup>I-LDL binding were determined by subtracting values for nonspecific binding from the values for total binding. The values for nonspecific binding represents the amount of <sup>125</sup>I-LDL bound to the monolayers in the presence of the 60-fold excess of unlabelled LDL. All values represent the average of data obtained from duplicate monolayers.

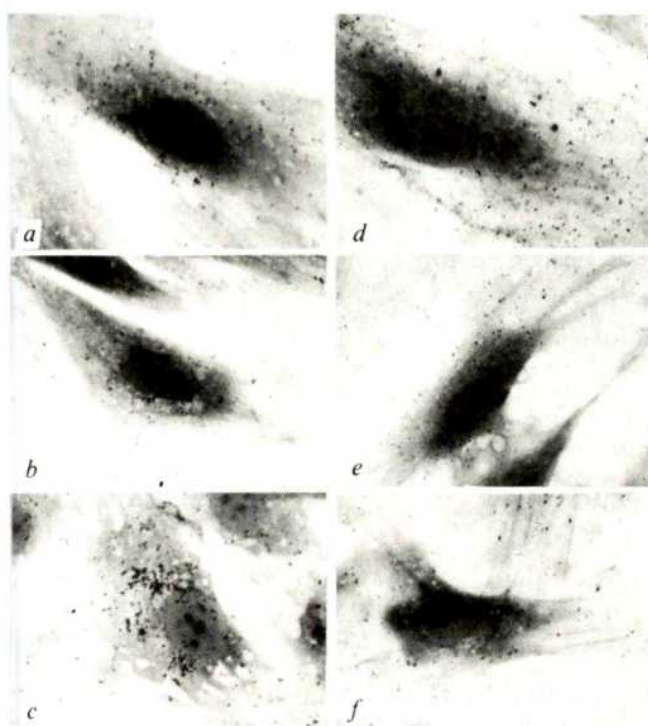
arrows) were bound in a random fashion at intervals along the plasma membrane remote from the coated pits.

### Quantitative analysis of LDL-ferritin binding

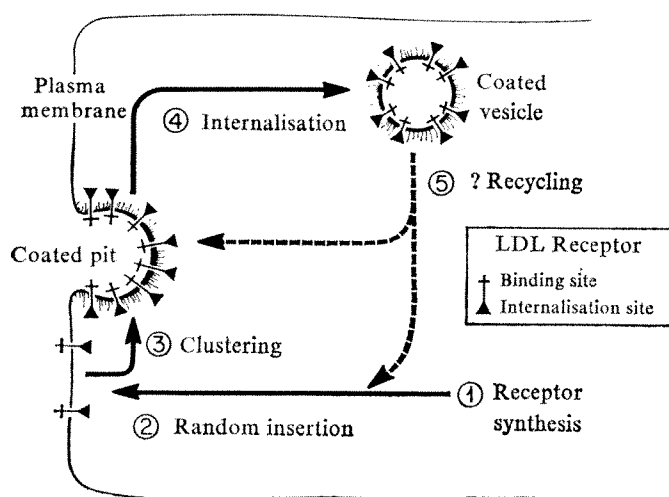
To confirm these qualitative findings by quantitative methods, fibroblasts from two normal subjects (+/+), from J.D. (R<sup>b</sup>/R<sup>b+.</sup>), and from J.D.'s heterozygous father (+/R<sup>b+.</sup>) were incubated with LDL-ferritin at 4 °C, washed extensively to remove nonspecifically bound material, and fixed in monolayer culture. Sections of the cells were prepared and examined at random by an observer who was unaware of the source of the specimens and the experimental design. Linear segments of plasma membrane totalling 4 mm in length were examined and the number of LDL-ferritin particles localised within coated pits or found on non-coated regions was recorded. As a part of the same experiment, one set of dishes from each genotype was incubated with <sup>125</sup>I-LDL and the amounts of total and high affinity <sup>125</sup>I-LDL binding to the LDL receptor were measured biochemically at 4 °C as previously described<sup>14</sup>. Table 1 shows that in the two normal cell strains (+/+), approximately 50% of the LDL-ferritin was bound over recognisable coated pits. In the J.D. cells (R<sup>b</sup>/R<sup>b+.</sup>), the total amount of LDL-ferritin binding (as well as that of <sup>125</sup>I-LDL) was only slightly lower than that in the normal cells, but only 3% of the LDL-ferritin particles were found in coated pits. In the cells from J.D.'s father (+/R<sup>b+.</sup>), the total amount of LDL-ferritin binding (as well as that of <sup>125</sup>I-LDL) was greater than that in the normal cells (Table 1). But, the percentage of LDL-ferritin bound in coated pits was only half as much as in normal cells (20% compared with 50%). This latter ultrastructural finding is consistent with previous biochemical data demonstrating that the cells of J.D.'s father contain two populations of LDL receptors: one population (the product of the normal allele) that internalises LDL normally<sup>13</sup> and is presumably incorporated normally into coated pits and a second population (the product of the R<sup>b+.</sup> allele) that fails to internalise receptor-bound LDL<sup>13</sup> and, in the current studies, seems to be excluded from the coated pits.

The quantitative and qualitative electron microscopic observations indicate that the defective internalisation of LDL in the J.D. cells is due to an inability of the LDL receptors specified by the R<sup>b+.</sup> allele to localise in coated pits. This conclusion was supported by additional experiments in which the disappearance of LDL-ferritin from the cell surface was quantified. In one of these experiments, normal cells and J.D. cells were incubated with LDL-ferritin at 4 °C for 2 h, washed, and then warmed to 37 °C for varying intervals before fixation. At each interval, the number of LDL-ferritin particles remaining on the cell surface was counted as previously described<sup>4,6</sup>. In the normal cells the number of surface-bound LDL-ferritin particles declined by 75% after the cells had been warmed for only 2 min. As previously

reported<sup>4</sup>, this disappearance of LDL-ferritin from the surface of normal fibroblasts is attributable to the internalisation of the lipoprotein in coated endocytic vesicles. In contrast to the results in the normal cells, the amount of LDL-ferritin bound to the



**Fig. 2** Autoradiograms of normal (a-c) and J.D.'s (d-f) fibroblasts exposed to <sup>125</sup>I-LDL for 2 h at 4 °C and then warmed to 37 °C for various times. Monolayers of fibroblasts were prepared in 60 × 15 mm Petri dishes and incubated for 48 h in 5% lipoprotein-deficient serum as described in Fig. 1 except that the cells were grown on glass coverslips (22 × 22 mm) as previously described<sup>4</sup>. On the day of the experiment (day 7 of cell growth), monolayers were cooled to 4 °C for 30 min. The medium was removed and replaced with 2 ml of ice-cold growth medium containing 5% human lipoprotein-deficient serum and 10 µg protein ml<sup>-1</sup> of <sup>125</sup>I-LDL (276 c.p.m. per ng protein)<sup>24</sup>. The monolayers were incubated at 4 °C for 2 h, after which each dish was washed six times at 4 °C with an albumin-containing buffer as previously described<sup>14</sup>. Some sets of coverslips were fixed immediately (a and d), while others received 2 ml of growth medium containing 5% human lipoprotein-deficient serum and were warmed to 37 °C in the Petri dish for either 10 min (b and e) or 60 min (c and f) before fixation. The fixative was 6% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.3)<sup>4</sup>. The coverslips were prepared for autoradiography as previously described<sup>4</sup>. The exposure time was 2 weeks at 4 °C. Autoradiograms were examined with an American Optical Microstar 10 and photographed with a Polaroid camera. 1,000 ×.



**Fig. 3** Schematic illustration of the proposed pathway by which LDL receptors become localised to coated pits on the plasma membrane of human fibroblasts. The sequential steps in this process are as follows: (1) synthesis of LDL receptors on polyribosomes, (2) insertion of LDL receptors at random sites along non-coated segments of plasma membrane, (3) clustering together of LDL receptors in coated pits, (4) internalisation of LDL receptors as coated pits invaginate to form coated endocytic vesicles, and (5) recycling of internalised LDL receptors back to the plasma membrane.

surface of the J.D. cells declined by less than 5% even after 10 min of incubation at 37 °C.

### Localisation of LDL receptors by autoradiography

The failure of internalisation of LDL in the J.D. cells could also be demonstrated by light microscopic autoradiography using  $^{125}\text{I}$ -LDL. Figure 2a shows a normal fibroblast incubated for 2 h at 4 °C with  $^{125}\text{I}$ -LDL and then prepared for autoradiography. The  $^{125}\text{I}$ -LDL is distributed over the entire cell surface. In the J.D. cells the  $^{125}\text{I}$ -LDL was bound similarly (Fig. 2d). When the normal cells were warmed to 37 °C for 10 min (Fig. 2b) or 60 min (Fig. 2c), there was a progressive increase in the radioactivity over the perinuclear area. Previous studies have shown that this migration of silver grains reflects the internalisation of the  $^{125}\text{I}$ -LDL and its transport to cellular lysosomes<sup>4</sup>. In contrast, when the J.D. cells were warmed for 10 min (Fig. 2e) or 60 min (Fig. 2f), there was no increase of the  $^{125}\text{I}$ -LDL in the perinuclear region. There was a slight reduction in the number of silver grains over the periphery of the J.D. cells with time at 37 °C (compare Fig. 2d and f). Previous biochemical measurements have shown that this is due to a slow dissociation of intact  $^{125}\text{I}$ -LDL from the receptor sites at 37 °C<sup>10</sup>.

### Implications for the mechanism of interaction of cell surface receptors with coated pits

Our results support the concept that the coated pit is a specialised region of the plasma membrane whose function is to undergo internalisation at a rapid rate and to carry receptor-bound molecules into the cell. The LDL receptors specified by the  $R^{b+}$  allele lack the ability to become localised within these coated pits. As a result, when LDL binds to these mutationally altered receptors, it is not carried into the cell and remains bound to the cell surface over non-coated regions of the plasma membrane. The mislocation of the mutant receptors specified by the  $R^{b+}$  allele can be demonstrated conveniently in the cells of J.D. ( $R^b/R^{b+}$ ) because the partner allele at the LDL receptor locus in J.D. is also mutant ( $R^b$ ), specifying a receptor that does not bind LDL<sup>13</sup>.

The manifestations of the  $R^{b+}$  mutation suggest that the incorporation of LDL receptors into coated pits may be analogous to the process by which membrane proteins of certain lipid-containing viruses are inserted into patches of host plasma membrane before the budding of the virus from the host cyto-

plasm<sup>15</sup>. In one of the best studied of these viral systems, that of vesicular stomatitis virus, the viral surface glycoprotein, G, is a transmembrane protein that is synthesised on host membrane-bound polyribosomes, glycosylated, and is then inserted at random into the host plasma membrane<sup>15-17</sup>. These G proteins are then clustered together as a patch in association with another viral protein, M, which is synthesised in the cytoplasm and coats the cytoplasmic side of the plasma membrane. Under the direction of the M protein, the nucleocapsid of the virus attaches to the patch of viral proteins and the plasma membrane then buds off to form a complete viral particle. In the process of coalescence of the G and M proteins, the host cell's own plasma membrane proteins are excluded from the viral patch, so that the mature viral membrane contains mainly virus-coded proteins<sup>15-17</sup>.

Figure 3 depicts a proposed working model for the incorporation of LDL receptors into coated pits that is based on the above model for viral membrane formation and on current concepts of membrane structure<sup>18,19</sup>. The LDL receptor is envisaged as a transmembrane protein that is synthesised on membrane-bound polyribosomes and inserted at random sites in the plasma membrane, probably after glycosylation in the Golgi apparatus. The external portion of the LDL receptor contains the binding site for LDL. The cytoplasmic portion contains a specific amino acid sequence that is necessary for recognition of the receptor as a component of coated pits. After their insertion into the plasma membrane, the receptor molecules that contain the proper recognition sequence are gathered together and incorporated into coated pits under the influence of unknown peripheral membrane proteins that interact at the cytoplasmic surface of the membrane. It is possible that clathrin, the protein that has been identified as the major component of the cytoplasmic coat of coated vesicles<sup>20,21</sup>, functions in this capacity.

Several aspects of the model in Fig. 3 are worthy of emphasis. First, earlier data indicate that the clustering of LDL receptors into coated pits is not dependent on the binding of LDL<sup>4,6</sup>. Thus, when fibroblasts are fixed with formaldehyde before incubation with LDL-ferritin, the majority of receptor sites are still found within coated pits<sup>6</sup>. It therefore seems that the sequence of receptor insertion into the plasma membrane, clustering into coated pits, and internalisation proceeds continuously whether or not LDL is present. Second, kinetic data strongly suggest recycling of internalised LDL receptors back to the cell surface. Thus, although nearly all of the identifiable LDL receptors on the cell surface enter cells within 10 min, they are immediately replaced with an equal number of functionally active receptors<sup>9,10,14</sup>. This internalisation and replacement continues at an uninterrupted rate for at least 6 h even when protein synthesis is inhibited totally with cycloheximide (unpublished).

The model in Fig. 3 helps to explain the defect in the mutant receptor produced by the  $R^{b+}$  allele. We postulate that this receptor is defective in its cytoplasmic portion that contains the recognition site for incorporation into coated pits. As a result, these mutant receptors are not recognised by the cytoplasmic proteins that form coated pits, they remain scattered at random along the cell surface, and hence they fail to internalise LDL.

As stated above, it seems likely that coated pits contain receptors for other macromolecules in addition to LDL. For example, the kinetics of binding, internalisation, and lysosomal degradation of epidermal growth factor<sup>12</sup> and transcobalamin II<sup>22</sup> in human fibroblasts are identical with those of LDL. In each case, essentially all of the receptor-bound ligand enters the cell within 10 min. On the basis of the model proposed in Fig. 3, we hypothesise that the incorporation of each of these receptors into coated pits involves a mechanism similar to that proposed for the LDL receptor.

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1. Roth, T. F. & Porter, K. R. *J. Cell Biol.* **20**, 313-332 (1964).
2. Fawcett, D. W. *J. Histochem. Cytochem.* **13**, 75-91 (1965).



3. Friend, D. S. & Farquhar, M. G. *J. Cell Biol.* **35**, 357-376 (1967).
4. Anderson, R. G. W., Brown, M. S. & Goldstein, J. L. *Cell* **10**, 351-364 (1977).
5. Roth, T. F., Cutting, J. A. & Atlas, S. B. *J. Supramolec. Struct.* **4**, 527-548 (1976).
6. Anderson, R. G. W., Goldstein, J. L. & Brown, M. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2434-2438 (1976).
7. Goldstein, J. L., Brown, M. S. & Anderson, R. G. W. in *International Cell Biology 1976-1977* (eds Brinkley, B. R. & Porter, K. R.) 639-648 (Rockefeller University Press, New York, 1977).
8. Brown, M. S. & Goldstein, J. L. *Science* **191**, 150-154 (1976).
9. Goldstein, J. L. & Brown, M. S. *Curr. Top. cell. Reg.* **11**, 147-181 (1976).
10. Brown, M. S. & Goldstein, J. L. *Cell* **9**, 663-674 (1976).
11. Brown, M. S. & Goldstein, J. L. *N. Engl. J. Med.* **294**, 1386-1390 (1976).
12. Carpenter, G. & Cohen, S. *J. Cell Biol.* **71**, 159-171 (1976).
13. Goldstein, J. L., Brown, M. S. & Stone, N. J. *Cell* **13**, 629-641 (1977).
14. Goldstein, J. L., Basu, S. K., Brunschede, G. Y. & Brown, M. S. *Cell* **7**, 85-95 (1976).
15. Lenard, J. & Compans, R. W. *Biochim. biophys. Acta* **344**, 51-94 (1974).
16. Knipe, D. M., Lodish, H. F. & Baltimore, D. *J. Virol.* **21**, 1121-1127; 1140-1148 (1977).
17. Knipe, D. M., Baltimore, D. & Lodish, H. F. *J. Virol.* **21**, 1128-1139; 1149-1158 (1977).
18. Bretscher, M. S. & Raff, M. C. *Nature* **258**, 43-49 (1975).
19. Singer, S. J. & Nicolson, G. L. *Science* **175**, 720-731 (1972).
20. Pearse, B. M. F. *J. molec. Biol.* **97**, 93-98 (1975).
21. Pearse, B. M. F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1255-1259 (1976).
22. Youngdahl-Turner, P., Allen, R. H. & Rosenberg, L. E. *Clin. Res.* **25**, 472A (1977).
23. Brown, M. S., Dana, S. E. & Goldstein, J. L. *J. biol. Chem.* **249**, 789-796 (1974).
24. Brown, M. S. & Goldstein, J. L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 788-792 (1974).
25. Onei et al. *Exp. Cell Res.* (In the press).

# letters to nature

## Evolution of dynamical systems with time-varying gravity

VARIOUS attempts have been made to place limits on the time-variability of gravity through observations of astrophysical objects. We demonstrate that contrary to previous studies<sup>1,2</sup>, no useful limits can be obtained from observations of  $N$ -body systems such as galaxies and star-clusters.

Van Flandern<sup>3</sup> has analysed over 21 years of lunar occultation data while Muller<sup>4</sup> has studied ancient astronomical observations of eclipses from 1374 BC, to AD 1715. The results of these two independent experiments can be combined for cosmologies which conserve angular momentum to imply<sup>4</sup>

$$G/G = -2.6 \pm 1.5 \times 10^{-11} \text{ yr}^{-1} \quad (1)$$

where  $G$  is Newton's gravitational 'constant'. In addition, there are several recent field theories involving a time dependent  $G$  which are compatible with both general relativity and all Solar System tests of relativity<sup>5,6</sup>.

In order to test these theories and explore the consequences of the above mentioned measurements, astrophysical effects of a time-dependent  $G$  must be examined in old astronomical objects that are dominated by gravity. For example, a recent analysis<sup>7</sup> of the spindown of pulsars with varying  $G$  placed a limit on the possible weakening of gravity which is just compatible with equation (1).

In another effort to test varying  $G$ , a numerical study of the dynamics of clusters of galaxies and globular clusters was made which found<sup>1</sup> that  $-G/G < 4 \times 10^{-11} \text{ yr}^{-1}$ . In this letter we study the evolution of dynamical systems with time varying gravity and show that these calculations as well as similar studies<sup>2</sup> are incorrect. We also demonstrate rigorously that, unlike the case of pulsar spindown, the dynamics of clusters of galaxies and globular clusters cannot place any useful limits on  $G/G$ .

For convenience we can, without loss of generality, keep all masses constant, let only  $G$  depend on time, and measure time on atomic clocks. Then consider a system of point masses,  $m_i$ , with trajectories  $\mathbf{R}_i(t)$ . The (time-dependent) gravitational constant is  $G(t) = G_0 a^{-1}(t)$ ;  $G_0 \equiv G(0)$  with  $t=0$  denoting the present epoch. The equation of motion of the  $i^{\text{th}}$  particle is, in the limit of Newtonian mechanics,

$$\frac{d^2 \mathbf{R}_i}{dt^2} = \sum_{j \neq i} G(t) m_j \frac{(\mathbf{R}_j - \mathbf{R}_i)}{|\mathbf{R}_j - \mathbf{R}_i|^3} \quad (2)$$

We define a new time coordinate  $\tau$  by

$$\tau(t) = \int_0^t a^{-2}(t') dt' \quad (3)$$

We describe the trajectories by  $\mathbf{r}_i$ , defined by  $\mathbf{R}_i(t) = \mathbf{R}_c(t) + a(t)\mathbf{r}_i(t)$ , where  $\mathbf{R}_c$  is the centre of mass of the system. The equation of motion now becomes

$$\frac{d^2 \mathbf{r}_i}{d\tau^2} = \sum_{j \neq i} G_0 m_j \frac{(\mathbf{r}_j - \mathbf{r}_i)}{|\mathbf{r}_j - \mathbf{r}_i|^3} - \mathbf{r}_i a^3 \frac{d^2 a}{dt^2} \quad (4)$$

The last term in equation (4) is zero if  $a(t)$  takes the usual form  $a(t) = (t+T)/T$ , where  $T$  is the age of the universe in atomic time as predicted by a given cosmology. When our results are specialised to the two-body problem using this form of  $a(t)$ , we recover Vinti's<sup>8</sup> solution. For an  $N$ -body system with a more general form of  $a(t)$  we define  $t_G \equiv G/(dG/dt)$  ( $t_G \approx T$  for cosmologies of interest) and define  $t_D$  as the dynamical time of the system. We then note that the relative importance of the last term in equation (4) is roughly

$$\frac{d^2 \mathbf{r}_i/d\tau^2}{r_i a^3 (d^2 a/dt^2)} \sim \frac{r_i \tau_D^2}{r_i a^4 (d^2 a/dt^2)} \sim t_G^2/t_D^2 \quad (5)$$

Thus, for any system in which  $t_D \ll t_G \approx T$  (the condition required for virial equilibrium) the system evolves precisely as it would if  $G$  were constant, except that its size and time scales are proportional to  $a(t)$  and  $a^2(t)$ , respectively. Thus, the 'qualitative' dynamical evolution of such equilibrium objects as galaxies, clusters of galaxies, and globular clusters is not affected by a time varying gravity in the Newtonian limit.

The homologous evolution of equilibrium gravitational systems described above is observable, in principle, because expansion (or contraction) would increase the r.m.s. velocity of the system by about  $r/t_G$  over the virial equilibrium value. The residual velocities would characteristically be proportional to distance from the centre of the system. But only when  $t_D \ll t_G \approx T$  may we invoke the virial theorem. In such cases the residual velocities are much smaller than the r.m.s. velocities. Therefore, the virial discrepancy caused by a time-variable gravity would probably be unobservable, even if a system's mass could be measured independent of the virial theorem. Lewis<sup>2</sup> recognised that a time-variable gravity creates a virial discrepancy. But he applied this idea to loose associations of galaxies in which  $t_D$  is not small compared to  $t_G$  and for which the virial theorem may not be appropriate.

Our results contradict the work of Dearborn and Schramm<sup>1</sup>. Their numerical results showed that individual members of an  $N$ -body system could escape as  $G$  decreased. Their scheme, however, ignored the evolution of the system as a whole, while following the orbits of single test particles. Thus, they were unable to recognise the homologous development of the system.

The present results do not apply to the latest version of the Dirac cosmologies<sup>5</sup> since then equation (2) has an additional cosmological term which may produce qualitative changes in the system over cosmological time scales.

The current properties of collisionless equilibrium systems (such as galaxies) cannot be used to place limits on  $G$  because, as

we have shown, they undergo no qualitative evolution. Observations of systems where two-body relaxation is important, such as globular clusters, can in principle provide such limits because they do undergo qualitative changes with time. But neither observations of globular clusters nor the theory of dynamical cluster evolution are currently precise enough to produce limits comparable to those provided by experiments. We should emphasise that local laboratory and Solar System tests of  $\dot{G}$  are quantitative in nature and not subject to the limitations inherent in qualitative dynamical tests.

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1. Dearborn, D. S. & Schramm, D. N. *Nature* **247**, 441–443 (1974).
2. Lewis, B. M. *Nature* **261**, 302–304 (1976).
3. Van Flandern, T. C. *Mon. Not. R. astr. Soc.* **170**, 333–342 (1975).
4. Müller, P. M. *Jet Propulsion Lab. Res. Report* (15 Sept. 1976).
5. Canuto, V., Adams, P. J., Hsieh, S.-H. & Tsaiang, E. *Phys. Rev. D* (in the press).
6. Bekenstein, J. D. *Phys. Rev. D* **15**, 1458–1468 (1977).
7. Mansfield, V. N. *Nature* **261**, 560–562 (1976).
8. Vinti, J. P. *Mon. Not. R. astr. Soc.* **169**, 417–427 (1974).

## Are supernovae sources of presolar grains?

ISOTOPIC measurements<sup>1</sup> in chondritic meteorites have revealed that live <sup>26</sup>Al was present in the early solar system. <sup>26</sup>Al and other reported isotopic peculiarities<sup>2–4</sup> in the meteorite Allende could be attributable to explosive nucleosynthesis, sparking suggestions<sup>1–8</sup> that a nearby supernova may have contaminated the early solar nebula. One explanation for isotopic anomalies in early nebular condensates is the injection of supernova grains. An advantage of this mechanism is that it could avoid excessive dilution of the anomalies by the bulk of the nebula. We examine here the early expansion of supernovae to determine the likelihood of grain nucleation and growth.

Models<sup>9–12</sup> of type II supernovae (SN II), consisting chiefly of an extended low density supergiant envelope, have obtained good quantitative agreement with observationally determined<sup>13</sup> light curve time scales, absolute magnitudes and expansion velocities. The light curve is generated by the passage of a strong shock wave through the envelope. The shock passage through the underlying mantle region may cause the production of anomalous isotopic compositions during explosive processing<sup>14</sup> of these zones, so the evolution of the (hydrogen depleted) mantle is of major interest.

These models<sup>9–12</sup> suggest the observed decline in luminosity at about 50–70 d results from recombination in and growing transparency of the expanding envelope. The mantle material may then be exposed, and several cases of significant light after this decline have been observed<sup>15</sup>. The inferred photospheric radii and effective temperatures fall in the range  $R_{ph} \sim 1\text{--}3 \times 10^{14}$  cm,  $T_e \sim 3\text{--}5 \times 10^3$  K, in agreement with calculations of light curves which include material inside the envelope (ref. 11 and S.F. in preparation). Such models indicate mantle densities of  $3 \times 10^{-15}$  to  $10^{-13}$  g cm<sup>-3</sup> by the time cooling to the condensation temperatures ( $T_c \sim 1,000$  K) has occurred. Numerical nucleation calculations (J.L. and S.F., in preparation) which include mass conservation, expansion and cooling effects, and sputtering imply that grain formation occurs when  $\phi > \phi_0 \equiv n f_c \alpha = 10^6$  cm<sup>-3</sup>. Here  $\alpha$  is an effective sticking probability,  $f_c$  the number fraction of condensibles, and  $n$  the material number density. One condensing species has been assumed in these calculations, and no attempt to include subsequent coagulation, or chemical or temperature effects on  $\alpha$  has been made. These calculations suggest that

**Table 1** Model characteristics and predicted broadband fluxes at Earth

$t$ (yr)	$(^\circ\text{K})$	$T_g$ (K)	$R/10^{15}$ cm	$\tau$	$d^2 F_1^*(jz)$	$d^2 F_{10}^*(jz)$
2.5	1000	1000	1	23	3(–8)	3.5(–5)
3.2	750	750	1.35	24	1(–10)	3.6(–5)
4.5	470	500	2	26	1(–14)	2.3(–5)
8.5	220	250	4	5	—	3.0(–6)
10.5	195	200	5	2	—	2.6(–6)
20.5	100	100	10	<.2	—	5.1(–9)

\*Interval 0.9–1.2  $\mu\text{m}$ ,  $d$  in kpc.

†Interval 9–12  $\mu\text{m}$ .

virtually all the mantle (which has  $n f_c \sim 2\text{--}10 \times 10^6$  cm<sup>-3</sup>) may form grains a year or so after explosion.

The resulting size distributions are relatively flat and centred about sizes  $a \sim 5 \times 10^{-8} \phi / \phi_0$  cm. Further growth through condensation is halted by monomer depletion, rather than by declining density as previously suggested<sup>16</sup>, an assumption which led to  $a \sim 1\text{--}2 \times 10^{-5}$  cm. If post-nucleation growth by coagulation is important<sup>17</sup>, sizes of order  $a \sim 1\text{--}3 \times 10^{-6}$  cm are predicted if the physical parameters of ref. 16 are assumed. But it is possible that C/O < 1 in the bulk of this ejecta<sup>18</sup>, so graphite will not condense and silicate grains dominate. Using our estimate for  $\phi$ , the coagulation mechanism leads to  $a \lesssim 10^{-6}$  cm. Thus SN II seem likely sites for the growth of small grains, perhaps containing some isotopically anomalous compositions. Incidentally, these grains are almost certainly too small to trap any fission products of the superheavy nuclei which have been suggested as responsible for the Xe anomalies in Allende<sup>19,20</sup>.

The case for type I supernovae is not so favourable. These events exhibit light curves with a long-term exponential tail (up to 600 d, ref. 14) and seem to require a source of continuous heat or radiation input. No self-consistent mechanism for explaining these features in the absence of a strong continuum<sup>13</sup> has yet been proposed. One suggestion<sup>21,22</sup> is that  $\sim 0.1$  M of <sup>56</sup>Ni could supply this energy by radioactivity. In any case, the energy required to explain the light curve requires the ejecta to cool so slowly that the density will be too small for effective nucleation<sup>23</sup>, and some observations support these ideas<sup>24</sup>. The exclusion of type I's as nucleation sites is further strengthened by the suggestion (J. Scalo, in preparation) that sputtering by energetic electrons (Compton scattered by energetic <sup>56</sup>Co-decay  $\gamma$  rays or  $\beta^+$ 's) will be sufficient to impede nucleation.

Grain temperature and hence predicted infrared fluxes would, of course, be sensitive to the presence of a radiative source underlying the mantle itself, but in the absence of clear indications of the requirement of such sources in SN II light curves<sup>16–12,25</sup> we consider grain heating by inelastic collisions with thermal gas particles. The grain temperature  $\theta$  can be estimated by balancing this heat input with the radiation losses of a grain<sup>26</sup>,

$$\bar{Q}(\theta, a) \sigma \theta^4 \simeq 0.5 n \mu_{\text{H}} v_{\text{th}}^3$$

where  $\sigma$  is the Stephan constant,  $v_{\text{th}}$  the gas thermal velocity, and the Planck mean absorption efficiency  $\bar{Q} \ll 1$  for silicate grains in this size and temperature range<sup>27</sup>. Because  $\bar{Q}$  is small,  $\theta$  does not differ markedly from the gas temperature  $T_g(t)$ . Fluxes at earth at various infrared wavelengths can then be predicted by assuming blackbody radiance of an equivalent sphere of radius  $rt$  at the grain temperature:

$$F_e \equiv \sigma \theta^4 b_\lambda \chi_\lambda (rt/d)^2,$$

$$b_\lambda \equiv \int_{\Delta\lambda} B_\lambda(\theta) d\lambda / \int_0^\infty B(\theta) d\lambda$$

is the fraction of Planck emission over  $\Delta\lambda$  at temperature  $\theta$ ,  $d$  is the distance, and  $\chi_\lambda \equiv \min(1, \tau_\lambda)$  is a correction for small optical depths. We consider the adiabatic expansion of a  $3 M_\odot$  mantle,

with initial temperature, density and expansion velocity fit observationally inferred photospheric radii and effective temperatures<sup>1,5</sup>. Table 1 gives model characteristics and predicted broadband fluxes at Earth for the intervals 0.9–1.2  $\mu\text{m}$  and 9.0–12.0  $\mu\text{m}$ , for selected times in the first several years. No effect on the visual light curve is expected, as in the case of some novae<sup>2,8</sup>, because of the absence of a strong underlying continuum source. Ground based detection of fluxes of 60 mJy are possible at 10  $\mu\text{m}$  using a 60-inch telescope and an integration time of 10 min (A. Harper, personal communication). The predicted fluxes lie below this current limit for objects further away than about 10–20 kpc, implying that observations cannot provide conclusive tests of grain formation.

Grain survival in subsequent stages of SN remnant expansion up to encounter with the solar nebula is difficult to analyse without any clear model of such an interaction. Deceleration of the remnant as it sweeps up interstellar matter leads to the formation of a dense neutral shell with leading and trailing ionisation edges and associated ionising ultraviolet fluxes<sup>2,9</sup>, and rarefaction (reverse shock) waves may produce observed 1–2 keV X-ray fluxes<sup>3,0</sup>, but the radiation from such sources or heating by hot dilute remnant gas at these late stages has little disruptive effect on pre-existing grains<sup>3,1</sup>. Estimates of  $\theta$  from radiative heating by the ultraviolet shell or thermal bremsstrahlung X-rays suggest values of only 10 K or so. If cosmic ray acceleration occurs in the same objects before nebula encounter, the possibility for electron sputtering exists (J. Scalo, in preparation), though this coincidence cannot at present be established.

Thus grains produced in such ejecta from a supernova near to the early Solar System seem likely to have survived until such time as they would encounter the primitive solar nebula. Details of an injection mechanism are unknown, and grain survival would depend on the extent of turbulence, mixing and clumpiness due to fluid instabilities like the Rayleigh–Taylor instability, caused by the supernova shock acting on the presupernova mantle or envelope itself (R. Chevalier, paper presented at UCSC Supernova Workshop), by the blast wave acting on the solar nebula before encountering the ejecta, or in the actual encounter of ejecta with the nebula (S.H.M., in preparation).

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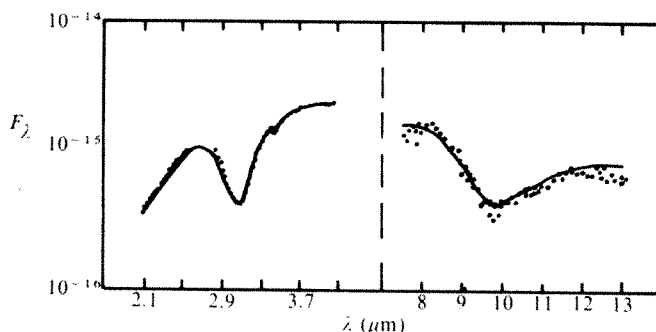
1. Lee, T., Papanastassiou, D. A. & Wasserburg, G. J. *Astrophys. J. Lett.* **211**, L107 (1977).
2. Black, D. C. *Geochim. cosmochim. Acta* **36**, 377 (1972).
3. Clayton, R. N., Onuma, N., Grossman, L. & Mayeda, T. K. *Earth planet. Sci. Lett.* **34**, 209 (1977).
4. Podosek, F. A. & Lewis, R. S. *Earth planet. Sci. Lett.* **15**, 101 (1972).
5. Clayton, D. D. *Nature* **257**, 36 (1975).
6. Sabu, D. D. & Manuel, O. K. *Nature* **262**, 28 (1976).
7. Cameron, A. G. W. & Truran, J. W. *Icarus* **30**, 447 (1977).
8. Lattimer, J. M., Schramm, D. N. & Grossman, L. *Nature* (in the press).
9. Grasberg, E. K., Imshennik, V. S. & Nadezhin, D. K. *Astrophys. Space Sci.* **10**, 28 (1971).
10. Arnett, W. D. & Falk, S. W. *Astrophys. J.* **210**, 733 (1976).
11. Chevalier, R. A. *Astrophys. J.* **207**, 872 (1976).
12. Falk, S. W. & Arnett, W. D. *Astrophys. J. Suppl.* **33**, (1977).
13. Kirshner, R. P., Oke, J. B., Penston, M. & Searle, L. *Astrophys. J.* **185**, 303 (1973).

14. Arnett, W. D. *A. Rev. astr. Astrophys.* **11**, 73 (1973).
15. Kirshner, R. P. & Kwan, J. *Astrophys. J.* **197**, 415 (1975).
16. Hoyle, F. & Wickramasinghe, N. C. *Nature* **226**, 62 (1970).
17. Simons, S. & Williams, I. P. *Astrophys. Space Sci.* **39**, 123 (1976).
18. Arnett, W. D. *On the Bulk Yields of Nucleosynthesis From Massive Stars* (EFI Preprint 77-26, University of Chicago, 1977).
19. Anders, E. & Heymann, D. *Science* **164**, 821 (1969).
20. Howard, W. M., Arnould, M. & Truran, J. W. *Astrophys. Space Sci.* **36**, L1 (1975).
21. Colgate, S. A. & White, R. N. *Astrophys. J.* **157**, 623 (1969).
22. Falk, S. W. *Bull. Am. Phys. Soc.* **22**, 87 (1977).
23. Lattimer, J. M., Schramm, D. N. & Grossman, L. *Astrophys. J.* (in the press).
24. Friedjung, M. *Astr. Astrophys.* **34**, 419 (1974).
25. Falk, S. W. & Arnett, W. D. *Astrophys. J. Lett.* **180**, L65 (1973).
26. Falk, S. W. & Scalo, J. M. *Astrophys. J.* **202**, 690 (1975).
27. Gilman, R. C. *Astrophys. J. Suppl.* **28**, 397 (1974).
28. Clayton, D. D. & Hoyle, F. *Astrophys. J.* **203**, 490 (1976).
29. Chevalier, R. A. *Astrophys. J.* **188**, 501 (1974).
30. McKee, C. F. *Astrophys. J.* **188**, 335 (1974).
31. Burke, J. R. & Silk, J. *Astrophys. J.* **190**, 1 (1974).

## Origin and nature of carbonaceous material in the galaxy

ASTRONOMERS generally believe that the carbonaceous material emerging from stars must be in the form of graphite, the most stable condensed form of carbon, and that such emergence must be confined to situations where the C/O ratio exceeds unity, such as in the atmospheres of carbon stars. We argue here that this state of affairs remains valid for mass flows from stars of sufficiently low surface temperatures, but it is not correct for low density flows from stars with colour temperatures  $\geq 4,000$  K (or for oscillatory stars with colour temperatures that go above 4,000 K for a portion of their cycle). In the latter case we show that carbonaceous material comprised mainly of polysaccharides will be able to condense.

We have previously discussed the likely presence of polysaccharides in galactic infrared sources<sup>1,2</sup>, and here we connect our discussion with the infrared model considered by Hoyle, Solomon and Woolf<sup>3</sup>. They considered that material flowed at rates  $\sim 10^{-2} M_{\odot}$  per year from out of O stars of high mass. In such a model, radiation from the star is absorbed and re-emitted by the outflowing material, which produces a shielding of the true surface of the underlying star. The temperature of the material falls with increasing distance from the star, until at a stage where the temperature is in the range  $\sim 5,000$ – $\sim 10,000$  K, an effective photosphere is formed. The effective photosphere develops at a radial distance  $r_0 \sim 10^{14}$  cm from the star, where the hydrogen density ( $n_H$ )<sub>0</sub> is of order  $10^{11}$  cm<sup>-3</sup> (for a mass flow of  $\sim 10^{-2} M_{\odot}$  yr<sup>-1</sup> at a speed of  $\sim 300$  km s<sup>-1</sup>). Further out in the flow molecular condensations occur, leading to the eventual absorption of radiation emitted by the effective photosphere of the star, and to the re-radiation of the absorbed radiation in the infrared. Would the resulting infrared emission from the model of Hoyle *et al.*<sup>3</sup> behave like the simpler polysaccharide models we considered previously<sup>1,2</sup>? This is answered by Fig. 1, which shows the expected emission from the Hoyle *et al.*'s model compared to observations of the BN source<sup>4,5</sup>. The expected emission was



**Fig. 1** Solid curve is the infrared emission for the source BN calculated for the model of Hoyle *et al.*<sup>3</sup>. The formation temperature of the polysaccharide grains was taken to be 850 K, and the grain temperature was taken to vary subsequently as the inverse square root of the distance from the exciting star. The optical depth of the region of formation of the grains was four times that of the sample of cellulose which gave rise to the transmittance values of Table 1 of ref. 2. The points represent observational data<sup>4</sup>.



calculated for a polysaccharide formation temperature  $T_m = 850$  K and for the relative transmittance values given in Table 1 of ref. 2. The radial opacity through the source was taken to be four times the relative values given in the latter table.

The new calculation is an improvement on our previous work in that no *ad hoc* assumption of a surface emitting like a black body has been made. This improvement, together with the agreement between theory and observation shown in Fig. 1, gives a strong empirical indication that polysaccharides can form in the mass flows from stars where the C/O ratio is probably less than unity. To give plausibility to such a view, three questions need answering: (1) Why is C not almost entirely consumed in the formation of CO? (2) Why is C not built into graphite rather than into polysaccharides? (3) In view of the inevitably small concentrations of the molecules  $C_2$ ,  $C_3$ , how can the bulk of the carbon manage to condense at all?

The answer to question (1) turns on three points. The ratio CO/C at the effective photosphere is essentially thermodynamic, less than  $\sim 10^{-6}$ . For a constant outflow speed, the hydrogen density  $n_H$  falls off according to

$$n_H = (n_H)_0 (r_0/r)^2 \quad (1)$$

With  $(n_H)_0 \simeq 10^{11} \text{ cm}^{-3}$ , and for a time scale of only a few years available for the recombination of atomic species into molecules, triple collisions of the kind  $A + B + H \rightarrow AB + H$  play no significant role in the formation of diatomic molecules, which must accordingly proceed through two-body radiative recombinations of the type



With a low cross-section  $\sim 10^{-22} \text{ cm}^2$  for  $C + O \rightarrow CO + h\nu$  and for a gas thermal velocity  $\sim 10^5 \text{ cm s}^{-1}$  the fraction CO/C built in the available time scale of  $10^7$  s is less than  $\sim 10^{-2}$ .

To answer question (2), we note that the internal energy of a large polyatomic molecule distributes itself statistically among the many states of the molecule, and it is appropriate to characterise the distribution by an internal temperature,  $T_m$ . The value of  $T_m$  for a particular molecule is determined by the equation of energy balance between the absorption of radiation from the star and the re-emission of radiation by the particular molecule, an equation of the form

$$(r_0/r)^2 \int_0^\infty Q_{\text{abs}}(\lambda) B_\lambda(T) d\lambda = 4 \int_0^\infty Q_{\text{em}}(\lambda) B_\lambda(T_m) d\lambda, \quad (3)$$

where  $B_\lambda$  is the Planck function. The factor 4 is for spherical particles, and it assumes no radiation field in the infrared. The presence of an infrared field reduces the factor 4, in a typical case to  $\sim 2$ . This reduction has no significant effect on the above argument since the reduction would apply to both the pyran ring and the graphite ring. Both the  $C_6$  ring which appears in graphite and the  $C_5O$  pyran ring which appears in the commonest polysaccharides absorb mainly in the ultraviolet with comparable values of  $Q_{\text{abs}}$ . But the pyran ring has a much higher value of  $Q_{\text{em}}$  in the infrared than the  $C_6$  ring, because the latter is a symmetric molecule with no dipole moment. Hence equation (3) leads to a significantly higher value of  $T_m$  for  $C_6$  than it does for  $C_5O$ , and this higher value of  $T_m$  more than offsets the greater binding energy of the  $C_6$  ring. (The binding energy difference is not large. The binding of the  $C_6$  ring from its constituent atoms is 30.5 eV and that for the  $C_5O$  ring is  $\sim 29$  eV (refs 6,7).

For question (3) begin by assuming the initial existence of a single polymer chain which we take to have been built through  $C_2, C_3$ . The logic will be that this first polymer chain, through rapid building interspersed by repeated fragmentation, can generate a vast cascade of further polymer chains which have nothing to do with the low concentrations of  $C_2, C_3$ . The logic is similar to that of the explosion of a nuclear weapon, where a first neutron is enormously amplified by the fission cycles which it provokes, and

where the flood of subsequent neutrons have nothing to do with the source of the first neutron.

For a flat chain of width  $D$ , length  $l$ , the number of carbon atoms arriving in a time  $dt$  which could lead to chain growth is

$$dN \simeq 2 n_c \langle v_{th} \rangle l D dt \quad (4)$$

[With  $n_0 \gtrsim n_c$ , the oxygen rate is moderately larger than equation (4).] Further, assuming a mean length interval between two successive polymer rings to be about  $2D$  and with six atoms making up a ring the increment of length corresponding to  $dN$  is

$$dl \simeq f(dN/6) 2D = (D dN/3) f, \quad (5)$$

where  $f$  is the fraction of impinging carbon atoms that diffuse and attach themselves to the ends of the polymer. Equations (4) and (5) give

$$l = l_0 e^{t/\tau_1} \quad (6)$$

with

$$\tau_1 = \left[ \frac{2}{3} n_c \langle v_{th} \rangle D^2 f \right]^{-1}. \quad (1)$$

The phenomenon of exponential growth we describe here is the same as that which occurs in the building of 'whiskers', which has been studied in the laboratory<sup>8,9</sup>. For  $n_c \simeq 10^6 \text{ cm}^{-3}$ ,  $D \simeq 5 \times 10^{-8} \text{ cm}$  and  $\langle v_{th} \rangle \simeq 10^5 \text{ cm s}^{-1}$ , therefore we obtain  $\tau_1 \simeq 2 \times 10^3 f^{-1} \text{ s}$ , and this is small compared to the available time scale of the order of a year, even for fractions  $f$  as low as  $10^{-2}$ . Ample time is therefore available for many exponential 'cycles', again in analogy to the many fission cycles of a nuclear weapon.

We expect polymers to appear in the mass flow from a star when the temperature  $T_m$  first becomes low enough for the bond linkages in the polymer to assume stability. For C-O-C linkages of a polysaccharide, the bond strength is  $\sim 4$  eV and the largest values of  $T_m$  for which such a bond will be stable lies in the range 800–900 K, just the polysaccharide formation temperature used in the calculations leading to Fig. 1.

The first polymers built through  $C_2, C_3$ , will be of short lengths and for them the value of  $T_m$  determined by equation (3) is less than for longer polymers with lengths  $l \simeq \lambda_{\text{star}}/2\pi$ . This is because a tuning effect appears as the polymers grow to a length that is resonant with the main optical radiation of the star,  $\lambda_{\text{star}} \simeq 5 \times 10^{-5} \text{ cm}$ . The polymers then experience a strong radiation pressure force due to scattering which gives them an appreciable drift velocity with respect to the ambient gas. Hoyle *et al.* estimated a drift velocity between 10 and 100  $\text{km s}^{-1}$  for their model. At 100  $\text{km s}^{-1}$  the viscous drag would raise the temperature of the polymer chain by about 100 K. We think even stronger heating than this could occur. Yet even 100 K is sufficient in a marginally stable situation to begin breaking the C-O-C linkages of the polymer. The reduced lengths of the fragments destroys the resonance, so that the fragments quickly assume lower values of  $T_m$ , thereby returning to a stable condition. Because of the explosive exponential growth implied by equation (6), the fragments almost immediately go through the same sequence as the original polymer. Not only do the lengths of the polymers grow exponentially but the numbers of the polymers also increase dramatically.

There is, of course, a limit to the number of particles that can be produced from a single first polymer chain, but the limit turns out to be very large. The limit arises because a first polymer chain, together with all its progeny, does not have access to all the carbon present in the whole mass flow. But there will be access at least to all the carbon within the distance through which carbon atoms can diffuse in the time scale of  $\sim 10^8$  s required for a given sample of material to flow from the star out to distances at which the density

falls off significantly. Thus the carbon diffusion distance in a time  $t$  is given by

$$\sim [\langle v_{th} \rangle t / \sigma_c n_H]^{1/2}$$

where  $\sigma_c$  is the cross-section for collisions between carbon atoms and hydrogen atoms. With  $t \approx 10^8$  s,  $\langle v_{th} \rangle \approx 10^5$  cm s<sup>-1</sup>,  $\sigma_c = 10^{-16}$  cm<sup>2</sup>,  $n_H \approx 10^{10}$  cm<sup>-3</sup>, the diffusion distance is  $\sim 3 \times 10^9$  cm. Thus the zone of influence of an initial polymer is at least  $(3 \times 10^9)^3$  cm<sup>3</sup>. It is actually greater than this because the polymers are subject to radiation pressure which causes a relative motion in the radial direction with respect to the gas. Using the relative velocity, 100 km s<sup>-1</sup> by Hoyle *et al.* the radial drift with respect to the gas developed by a polymer in the time scale  $t \sim 10^8$  s is as large as  $10^{15}$  cm. Thus the zones of influence of initial polymers are cylindrical in shape with axes of length  $\sim 10^{15}$  cm along the radial direction from the star and with transverse dimensions of  $\sim 3 \times 10^9$  cm, the latter being the carbon atom diffusion distance calculated above. The zone of influence of a single starting short polymer chain, therefore, has volume of order  $10^{15} (3 \times 10^9)^2 \approx 10^{34}$  cm<sup>3</sup>, compared with a total volume of  $\sim 10^{45}$  cm<sup>3</sup> for the effective condensation region of the whole mass flow. Only some  $10^{11}$  starting polymers are needed to mop up the whole of the carbon,  $10^{11}$  starting polymers in  $10^{45}$  cm<sup>3</sup> giving a space density of  $10^{-34}$  cm<sup>-3</sup>.

The required formation rate through C<sub>3</sub>C<sub>3</sub> of initial short polymer chains therefore takes the exceedingly low value of  $\sim 10^{-38}$  cm<sup>-3</sup> s<sup>-1</sup>. The rates of formation of C<sub>3</sub>C<sub>4</sub> by C<sub>2</sub> + C → C<sub>3</sub>, C<sub>3</sub> + C → C<sub>4</sub> are uncertain, but even with low cross-sections for both reactions, and even with ample allowance for the destruction of C<sub>2</sub> through C<sub>2</sub> + O → CO + C, a fraction  $\sim 10^{-12}$  of all the carbon will become C<sub>4</sub> in the available time scale. The molecule C<sub>4</sub> already has a sufficient number of internal states for further additions to occur with comparatively large cross-sections,  $\sim 10^{-16}$  cm<sup>2</sup>. Thus beyond C<sub>4</sub> the apparent difficulty of small formation rates disappears, and the number of initial polymer chains that could be achieved (if necessary) could be comparable to the number of C<sub>4</sub> nuclei, which is very many orders of magnitude greater than the minimum required number of initial short polymer chains. The first such chains to flow through C<sub>2</sub>C<sub>3</sub>C<sub>4</sub>... therefore go on to take the whole of the carbon. This completes the answer to the third question.

If mass loss from a highly luminous O star proceeds for long enough, the oxygen-rich envelope will be replaced by material that has been processed in the star by the CN cycle. The oxygen will now be almost totally depleted, the carbon abundance will be reduced to about 1/10 of its normal value, and there will be a large excess of nitrogen. Under these conditions C<sub>4</sub> rings would evolve into heterocyclic C<sub>4</sub>N, C<sub>5</sub>N and C<sub>4</sub>N<sub>2</sub> rings. It may well be important in this connection that the porphyrins have a strong absorption band near 4,430 Å, the wavelength of a well-known interstellar absorption feature<sup>10</sup>, and that quinazoline and its derivatives have a strong absorption at 2,200 Å (see ref. 11) close to another interstellar feature.

Photospheric temperatures of evolved supergiant stars are uncertain. If mass flows in M and N-type stars originate in layers of the atmosphere where the colour temperature  $> \sim 4,000$  K, arguments similar to those discussed above would apply.

We conclude the following. Infrared sources exhibiting polysaccharide absorption features may be associated with massive stars of the kind discussed by Hoyle *et al.*<sup>3</sup>. Mass flows from such stars can lead to the production of polysaccharides in the first instance, followed by the condensation of nitrogenated heterocyclic carbon compounds. The composition of carbonaceous condensates from these sources may well determine the course of interstellar chemistry. Low molecular weight organic compounds and radicals detected in interstellar clouds, often involving a number of linked carbon atoms, are probably the products of the ultraviolet and cosmic-ray degradation of much more complex structures.

We also speculate that carbonaceous materials formed in the mass flows from stars provided the chemical basis for the origin of

life. It is not as hard as it has usually been supposed to understand how interstellar materials could have by-passed the destructive high temperature of the solar nebula and could have arrived undamaged at the Earth. We suggest that the origin of life on the Earth involved essentially the assembly of complex chemical components which had already been provided in the last stages of the accumulation of the Earth, and which exist widely and in comparatively great quantities within the interstellar medium.

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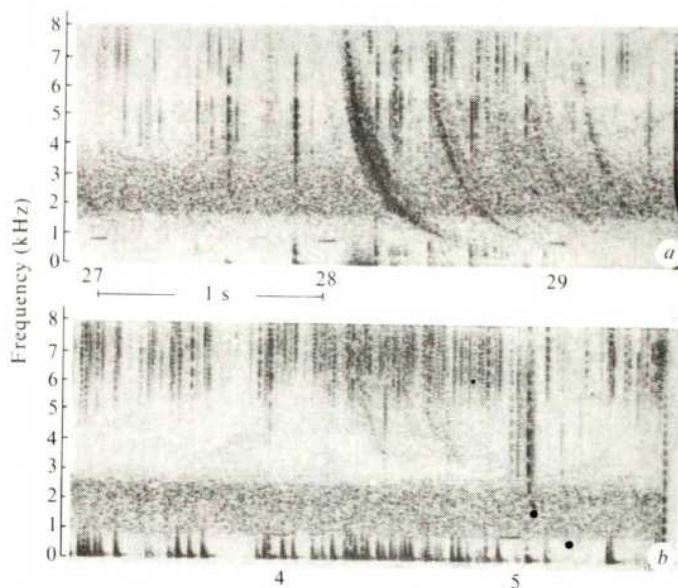
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1. Hoyle, F. & Wickramasinghe, N. C. *Nature* **268**, 610-612 (1977).
2. Hoyle, F. & Wickramasinghe, N. C. *Mon. Not. R. astr. Soc.* **181**, 51P (1977).
3. Hoyle, F., Solomon, P. M. & Woolf, N. J. *Astrophys. J.* **185**, L89 (1973).
4. Gillett, F. C. & Forrest, W. J. *Astrophys. J.* **179**, 483 (1973).
5. Merrill, K. M., Russell, R. W. & Soifer, B. T. *Astrophys. J.* **207**, 763 (1976).
6. Cox, J. D. *Tetrahedron* **19**, 1175 (1963).
7. Dewar, M. J. S. & Harget, A. J. *Proc. R. Soc. Lond. A* **315**, 442 (1970); **315**, 457 (1970).
8. Meyer, L. *Proc. 3rd Conf. Carbon* 451. (Pergamon Press, New York, 1959).
9. Donn, B. D. & Sears, B. W. *Science* **140**, 1208 (1963).
10. Johnson, F. M. *Ann. N.Y. Acad. Sci.* **194**, 3 (1971).
11. Albert, A. & Armarego, W. L. F. *Adv. Heterocyclic Chem.* **4**, 1 (1965).

## ELF emissions observed at Moshiri

MORPHOLOGICAL characteristics of ELF emissions (frequency 2-3 kHz) observed at a low latitude ground station of Moshiri in Japan (geomag. lat. 34.5°N;  $L = 1.59$ ) are reported here. ELF emissions in this frequency range are most poorly understood in the magnetospheric wave phenomena, and the information obtained on the ground will be of crucial importance in the study of their generation and propagation mechanisms. We have been observing VLF and ELF emissions at Moshiri<sup>1-3</sup> since 1964. We believe that Moshiri is at a lower latitude than any other where the emissions are continuously recorded<sup>4</sup>. Emissions of the hiss type are measured, by the minimum detection method<sup>1</sup>, at four specific

Fig. 1 Sonagrams of ELF emissions observed at Moshiri. *a*, 0950 LT 11 January 1976; *b*, 0750 LT 2 April 1976.



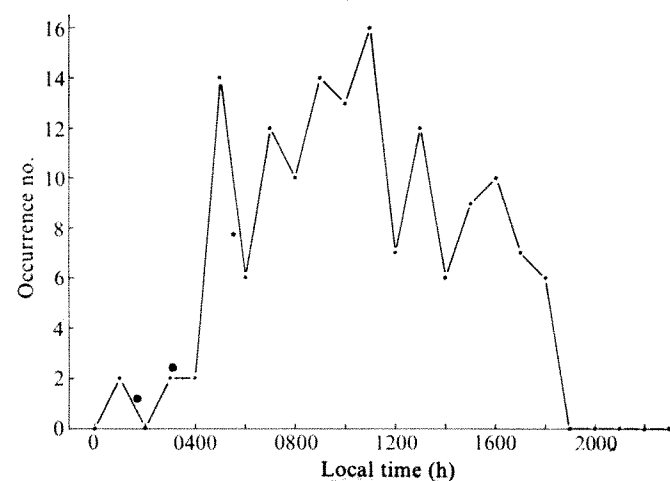


**Table 1** Characteristics of ELF emissions

	Ground observations	Satellite observations	
		Pogo (Kimura)	Isis (Ondoh)
Period of data	January 1974–December 1976	July 1968–May 1969	July 1970–April 1971
Diurnal variation	5–18 h LT	3–12 h LT	?
$K_p$ dependence	$K_p > 4$	higher $K_p$	$K_p = 1-3$
Frequency spectrum	2–3 kHz mainly	$2 \pm 0.5$ kHz	$< 3$ kHz
Latitudinal distribution	Two week cutoff: Yes, high latitude penetration No, low latitude penetration	Peak at high latitudes Wide latitude coverage	Peak at 50–60° Wide latitude coverage
$K_p$ -LT dependence	Shift to morning with increasing $K_p$	?	?
$K_p$ -frequency spectrum	No relation	?	Wider band with increasing $K_p$
LT-frequency spectrum	No relation	?	?

frequencies of 8 ( $\Delta f = \pm 1.25$  kHz), 5 ( $\pm 1$  kHz), 1.5 ( $\pm 0.1$  kHz) and 0.8 ( $\pm 0.1$  kHz) kHz, and recorded on paper charts. Wide band (0–10 kHz) observations on magnetic tapes have also been conducted following the synoptic schedule of two minutes every hour. We have found, using Ariel 3 VLF data, that the storm-time VLF emissions at Moshiri are generated around the plasmopause by the electron cyclotron instability with ring current electrons<sup>5</sup>. But the large number of VLF emissions appearing during magnetically quiet times have not been well studied<sup>2</sup>. Satellite measurements made on board Pogo (I. Kimura, personal communication) and Isis<sup>6</sup> have yielded a new type of ELF emissions ( $f < 3$  kHz).

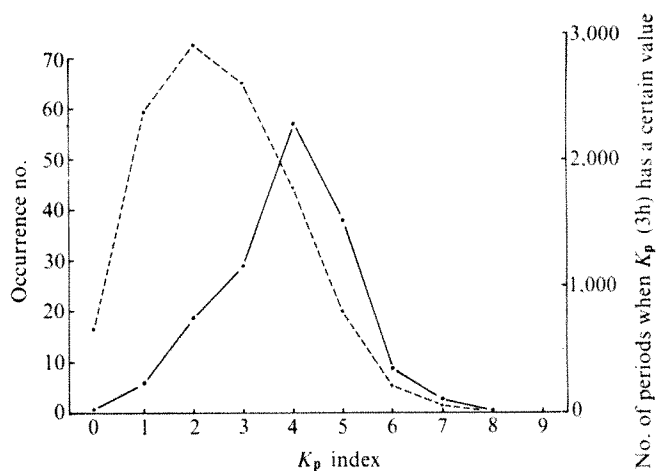
Here we describe the morphology of those ELF emissions observed at Moshiri based on magnetic tape records during four years from January 1973 to December 1976. During this period we identified about 160 ELF emission events. They were mainly of the hiss type, but we could find others including the chorus structure. Moshiri is located in an isolated village, free from artificial noises, and we could not detect any fine structure due to the power line harmonics radiation<sup>7,8</sup>. Hence, we consider that these ELF emissions are a natural phenomenon. Figure 1 shows the frequency spectra of ELF emissions; *a* is the sonagram of typical narrow band ELF emissions whose energy is concentrated in 2–3 kHz, and this frequency band is most often seen, whereas *b* shows lower frequency emissions (1–2.5 kHz). The low frequency

**Fig. 2** Local time dependence of occurrence number of ELF emissions.

cutoff frequency of ELF emissions is, on occasions, coincident with the cutoff frequency of the first order mode of the Earth-ionosphere waveguide propagation or two weeks. Examination of these sonagrams suggests that the frequency band of 2–3 kHz depends neither on local time nor  $K_p$  index, which is in good agreement with satellite results<sup>6</sup>.

Figure 2 shows the local-time dependence of the occurrence number, and indicates that these ELF emissions occur predominantly in the daytime (5–18 h LT). A comparison of local-time dependences for three ranges of  $K_p$  index shows that the occurrence number is concentrated in the afternoon during quiet periods  $K_p \leq 2+$ , but in the morning during moderate disturbances  $3- \leq K_p \leq 5+$ . Figure 3 shows the  $K_p$  dependence of occurrence number of ELF emissions (full line), and the number of periods when  $K_p$  (3 h) has a certain value (broken line). A comparison of the two curves indicated that the occurrence probability shows an abrupt increase from  $K_p = 4$ , and a broad maximum in the  $K_p$  range from 5 to 7, in good agreement with the satellite result. So, ELF emissions are likely to be associated with magnetic disturbances.

Assuming that the propagation under the ionosphere is roughly confined to the magnetic meridian plane<sup>9</sup>, ELF emissions with a low frequency cutoff, being associated with the two weeks cutoffs, are considered to have penetrated the ionosphere at latitudes higher than the station latitude, and followed the Earth-ionosphere waveguide mode of propagation<sup>10</sup>. There are also ELF emissions

**Fig. 3** Dependence on  $K_p$  index of occurrence number of ELF emissions and the number of periods in which  $K_p$  has a certain value.

whose lower cutoff frequencies extend well below the cutoff for the Earth-ionosphere waveguide mode propagation (see Fig. 1*b*). This indicates that these emissions seem to have emerged from the ionosphere near the station, with their wave normal directions in the ionosphere lying within the transmission cone<sup>11</sup>. These characteristics are not incompatible with the wide latitudinal coverage found by the satellites<sup>6</sup>. Table 1 summarises the general properties of ELF emissions based on our ground observations and on satellite results. Our initial impression is that these properties are similar to those of plasmaspheric hiss whose centre frequency is much lower,  $\sim 0.5$  kHz<sup>12–14</sup>.

The generation and propagation mechanisms of ELF emissions need further study. Their narrow band nature, however, suggests that the generation region may be very localised in the equatorial plane<sup>5</sup>. Considering that their occurrence is highly enhanced at 50–60° (ref. 6), the generation possibly occurs just within the plasmopause. If the waves are generated at various wave normal angles, some can propagate in complex non-ducted paths to the ionosphere over a wide latitude range<sup>6</sup> and, for favourable wave normal directions<sup>11</sup>, can be detected on the ground. The association of the occurrence of ELF emissions with magnetic storms or substorms is being investigated, and could give us insight into the



energetic particles in the outer radiation belt responsible for the excitation of ELF emissions.

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1. Iwai, A., Otsu, J. & Tanaka, Y. *Proc. res. Inst. Atmos.* **11**, 29–40 (1964).
2. Tanaka, Y., Hayakawa, M. & Ohtsu, J. *Rep. ionosph. Space Res.* **28**, 168–172 (1974).
3. Hayakawa, M., Tanaka, Y. & Ohtsu, J. *J. atmos. Terr. Phys.* **37**, 517–529 (1975).
4. Jorgensen, T. S. *J. geophys. Res.* **71**, 1367–1375 (1966).
5. Hayakawa, M., Bullough, K. & Kaiser, T. R. *Planet. Space Sci.* **25**, 353–368 (1977).
6. Ondoh, T., Aikyo, K. & Nagayama, M. *J. Radio Res. Labs. Jap.* **19**, 23–51 (1972).
7. Helliwell, R. A., Katsufurakis, J. P., Bell, T. F. & Raghuram, R. *J. geophys. Res.* **80**, 4249–4258 (1975).
8. Bullough, K., Tatnall, A. R. L. & Denby, M. *Nature* **260**, 401–403 (1976).
9. Iwai, A. & Tanaka, Y. *Proc. res. Inst. Atmos.* **15**, 1–16 (1968).
10. Otsu, J. *Proc. res. Inst. Atmos.* **7**, 58–71 (1960).
11. Hayakawa, M. & Iwai, A. *J. atmos. Terr. Phys.* **37**, 1211–1218 (1975).
12. Thorne, R. M., Smith, E. J., Burton, R. K. & Holzer, R. E. *J. geophys. Res.* **78**, 1581–1596 (1973).
13. Thorne, R. M., Church, S. R., Malloy, W. J. & Tsurutani, B. T. *J. geophys. Res.* **82**, 1585–1590 (1977).
14. Bullough, K. *et al. Proc. R. Soc. Lond. A* **343**, 207–226 (1975).

## A vitreous Kr-2SiO<sub>2</sub>

THERE are many studies<sup>1–7</sup> of the presence of inert gases in glasses. Because they have no electron affinity and high ionisation potentials (12 eV for Xe to 24 eV for He) the solubility of inert gases in glasses has been considered a simple physical process with no strong chemical interaction within the glass network. Recent work on pressure dependence of inert gas in vitreous<sup>1</sup> and crystalline<sup>2</sup> silica (SiO<sub>2</sub>) showed that the equilibrium solubility may be explained qualitatively by a statistical thermodynamic model<sup>3</sup>. At non-equilibrium conditions, however, a large quantity of inert gases can be incorporated into SiO<sub>2</sub> by processes such as glow discharge<sup>8,9</sup> and sputtering<sup>10,11</sup>, and here, the amount of inert gases in SiO<sub>2</sub> far exceeds the equilibrium solubility. Using a high-rate sputtering technique, we prepared a SiO<sub>2</sub> deposit containing 16.1 at.% Kr, expressed by a

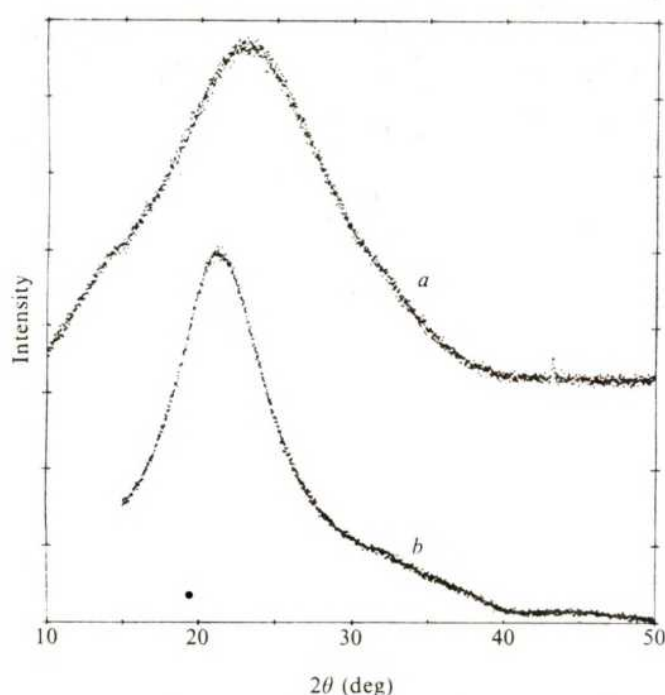


Fig. 1 X-ray diffraction patterns of the Kr-2SiO<sub>2</sub> deposit (a) and the target SiO<sub>2</sub> (b).

glass formula, Kr-2SiO<sub>2</sub> and we report here the unique characteristics of this material.

The Kr-2SiO<sub>2</sub> deposit was prepared by a modified r.f. sputtering apparatus<sup>12,13</sup> at a sputtering rate of 30 μm<sup>-1</sup>h. The target was a 12.7-cm-diameter, 0.64-cm-thick fused SiO<sub>2</sub> disk. Sputtering gas was krypton and oxygen 1:1 mixture; the addition of oxygen to the sputtering gas was required for stoichiometric-oxide formation. Deposition was onto a water-cooled copper substrate that had floating electrical potentials.

The Kr-2SiO<sub>2</sub> deposit was about 0.2 mm thick, and had the general appearance of a thin fused silica plate. Its

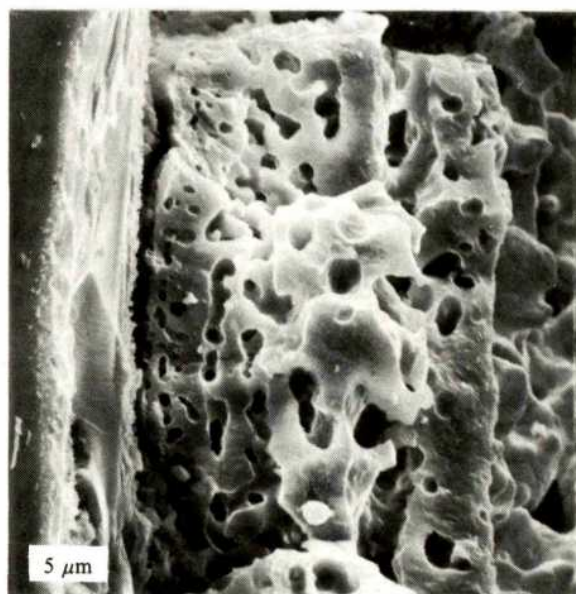


Fig. 2 Formation of the micropores after heating the Kr-2SiO<sub>2</sub> deposit to 1,000 °C.

surface was smooth, and contained a low density of macroscopic defects under ×100 magnification. A fracture surface along the thickness of the deposit resembled the fracture of an oxide glass.

The compositions of Kr and Si were determined by non-dispersive X-ray fluorescence analysis. The X-ray intensity of Kr was corrected for self-absorption assuming a uniform distribution of Kr throughout the deposit. It indicated a Kr/Si mole ratio of 0.57 ± 0.066, or 16.1 at.% Kr, from which the Kr-2SiO<sub>2</sub> formula was assigned.

By comparison with the X-ray diffraction pattern of the fused SiO<sub>2</sub> target the diffraction peak of the Kr-2SiO<sub>2</sub> deposit was nearly two times broader (Fig. 1). This indicated an increasingly disordered short-range atomic packing in the Kr-2SiO<sub>2</sub> deposit, possibly due to combined effects of high-rate sputtering and Kr entrapment, but the Kr-2SiO<sub>2</sub> had high thermal stability based on no weight loss observed after isothermal treatments at 700 °C for 2 h in air. Rapid release of Kr was observed during 1,000 °C annealing, and the sample became a popcorn-like flake containing 0.1 to 10 μm size pores (Fig. 2).

This may be the first time that such a high content of Kr has been incorporated into SiO<sub>2</sub>. It would be interesting to consider the role of Kr in the structure. Because the general appearance and the properties of the deposited Kr-2SiO<sub>2</sub> are similar to fused silica in many aspects<sup>13</sup>, we can assume that Kr atoms participate little in bonding with the network atoms, but are trapped in the vitreous SiO<sub>2</sub>. If Zachariasen's glass network model is assumed for the SiO<sub>2</sub> structure, we can see that those pores made by 4–7 oxygen atoms are too small to accommodate the Kr atom which has an atomic diameter of 3.99 Å and an atomic volume of 44.95 Å<sup>3</sup> at 0 K (ref. 14). As shown in Fig. 3,



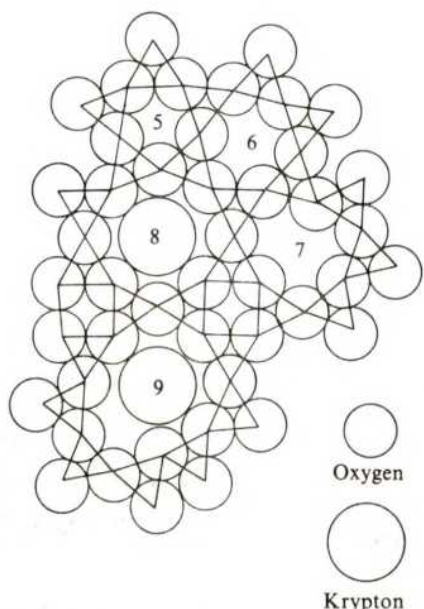


Fig. 3 Possible trapping of single Kr atom in the  $\text{SiO}_2$  network. Different pore sizes are shown by the number of oxygen atoms.

only pores made by eight or more oxygen atoms are big enough to trap the Kr atom. Since there are few such large pores (probably 10%) in the homogeneous structure model, the number of Kr atoms trapped cannot account for the Kr composition. We may consider two possible mechanisms for the Kr entrapment: (1) the  $\text{Kr-2SiO}_2$  consists of an  $\text{SiO}_2$  network similar to that of fused  $\text{SiO}_2$ ; a portion of Kr atoms is trapped individually in the pores made by eight or more oxygen atoms and a portion of Kr atoms is in the form of inhomogeneous clusters, and (2) the  $\text{Kr-2SiO}_2$  is composed of a distorted  $\text{SiO}_2$  network to accommodate individual Kr atoms.

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- Shelby, J. E. *J. appl. Phys.* **47**, 135 (1976).
- Barrer, R. M. & Vaughan, D. E. W. *Trans. Faraday Soc.* **63**, 2275 (1967).
- Shackelford, F. J., Studt, R. L. & Fulrath, R. M. *J. appl. Phys.* **43**, 1619 (1972).
- Doremus, R. H. in *Glass Science*, ch. 8 (Wiley, New York, 1973).
- Shelby, J. E. *J. Am. Ceram. Soc.* **55**, 61 (1972).
- Shelby, J. E. *Phys. Chem. Glasses* **13**, 167 (1972).
- Swets, D. E., Lee, R. W. & Frank, R. C. *J. chem. Phys.* **34**, 17 (1961).
- Grant, W. A., Carter, G. *Vacuum* **15**, 477 (1965).
- Young, J. R. *J. appl. Phys.* **27**, 926 (1956).
- Hoffmeister, W. & Zuegel, M. *Thin Solid Films* **3**, 35 (1969).
- Petersson, S., Linker, G. & Meyer, O. *Phys. Stat. Sol. A* **14**, 605 (1972).
- McClanahan, E. D., Busch, R., Fairbanks, J. & Patten, J. W., presented at Gas Turbine Conference and Products Show, Zurich, Switzerland, March 3–April 14, 1974.
- Wang, R. & Bayne, M. A., *Proceedings of Second American Nuclear Society Meeting on Fusion Technology*, p. 447 (1976).
- Donohue, J. *The Structure of Elements* ch. 2, 27 (John Wiley, New York, 1974).

## Anion deficiency in strontium titanate

STUDIES of the anion deficient phases of titanium and vanadium perovskites showed that the perovskite  $\text{SrTiO}_{3-x}$  has a wide range of composition, extending from  $x = 0$  to  $x = 0.5$  (ref. 1). The nonstoichiometry was inferred from the monophasic behaviour of the solid within this compositional range as obtained by means of powder X-ray diffraction. The same cubic structure was found for both  $\text{SrTiO}_{2.5}$  and  $\text{SrTiO}_3$ . Perovskites have been widely

studied recently in view of the very interesting and useful properties of these oxides<sup>2</sup>. The physicochemical properties of  $\text{SrTiO}_3$  include semiconductivity<sup>3,4</sup> and superconductivity<sup>5</sup> as well as peculiar phase transitions<sup>6</sup>. Of particular interest in this, and other<sup>7</sup>, systems is the presence of point defects and their influence on those properties. It seems that, at small degrees of reduction, doubly ionised oxygen vacancies are the main defects present on  $\text{SrTiO}_{3-x}$  (ref. 8). Although in that case, the oxygen vacancies, being at small numbers, are probably distributed at random, the presence of so many empty oxygen positions as the stoichiometry of  $\text{SrTiO}_{2.5}$  implies—one in every six oxygens missing—automatically suggests the possibility of ordering. This possibility seems to be realised under the synthesis conditions that we have used and we report here some of the results so far obtained in the system  $\text{SrO-Ti}_2\text{O}_3\text{-TiO}_2$ .

Samples of composition  $\text{SrTiO}_{3-x}$  with several values of  $x$  were prepared by heating appropriately weighted amounts of the parent oxides in quartz ampoules, sealed under vacuum, at 1,200 °C for 2 weeks. The ampoules were then quenched in liquid nitrogen. We have never obtained an homogeneous product but rather a compact powder with colours ranging from violet to yellow from the outside to inside. The X-ray data of a blue sample of nominal composition  $\text{SrTiO}_{2.5}$  could be interpreted as from a cubic unit cell with  $a = 3.900 \pm 0.003$  Å, in excellent agreement with the previously accepted value<sup>1</sup>. No superstructure lines were detected. Electron diffraction showed a very different situation, however. Figure 1 shows a pattern obtained on a Siemens 102 Elmiskop microscope fitted to a double tilting stage and operated at 100 KV. This pattern can be indexed as the  $[\bar{1}\bar{2}\bar{1}]$  zone axis of the same cubic X-ray subcell. There is a clear sixfold superlattice along  $^*111$  suggesting a long spacing of  $\sim 13.52$  Å. Figure 2 shows an electron micrograph of the same crystal area where the super-lattice fringes are resolved.

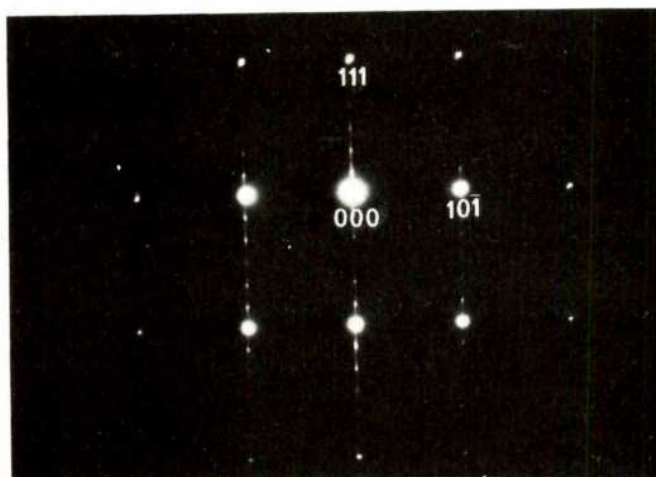


Fig. 1 Electron diffraction pattern of  $\text{SrTiO}_{2.5}$  showing a sixfold superlattice along  $^*111$  of the perovskite subcell. Zone axis  $[\bar{1}\bar{2}\bar{1}]$ .

The diffraction pattern is faintly streaked and it can be seen that the fringes are not equidistant, although their separation is often of  $\sim 13$  Å. On this particular sample the superlattice was always sixfold and lying on  $^*111$ . Figure 3 shows an even more interesting example. This pattern, from another crystal of the same blue powder, can be indexed on the  $[\bar{1}\bar{1}\bar{0}]$  zone axis of the cubic subcell. It can be seen that there are in fact two sixfold superstructures lying along  $^*111$  and  $^*1\bar{1}\bar{1}$ . Again there is streaking along those two directions. Although at first sight this pattern could be attributed to a twinned crystal the situation is somewhat more complicated as shown on the corresponding micrograph, Fig. 4. It can be seen that there are two sets of lattice fringes which cross at an angle of  $70.5^\circ$  as expected from the diffraction pattern. Even more interesting is the fact that the fringes intercross without apparent distortion and also that they are not always perfectly straight. On the other hand some of them, especially the set shown at B do end within the crystal and not at the crystal edge. The



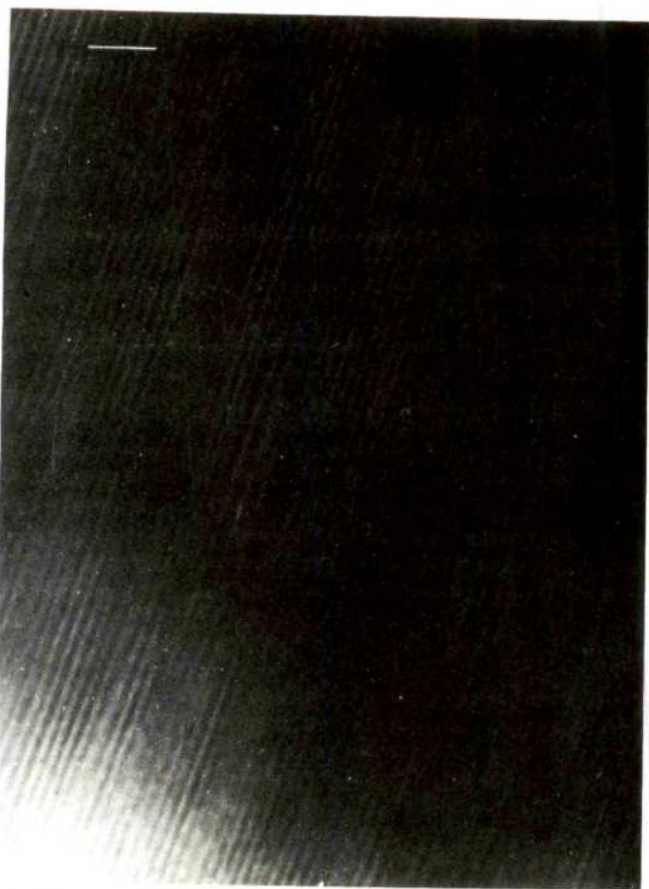


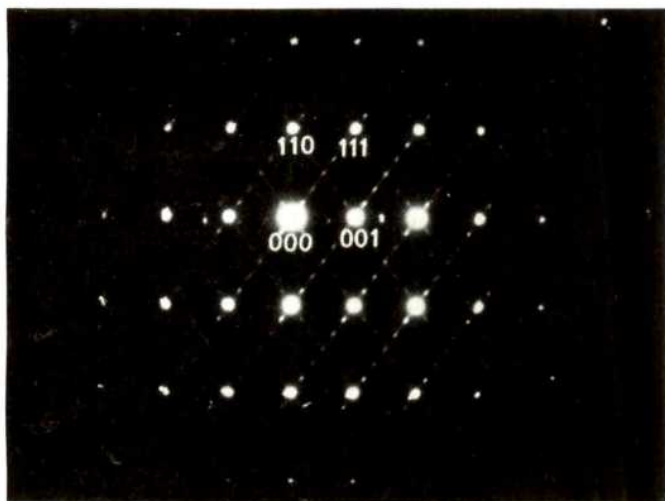
Fig. 2 Electron micrograph corresponding to Fig. 1 showing the superlattice fringes. Note that the fringe spacing is irregular.

Scale bar, 100 Å.

streaking is clearly understood in view of the uneven separation between the fringes in both sets.

The removal of an oxygen ion from a close-packed structure produces a drastic symmetry change in the environment of the metallic atom (or atoms) as in this case, coordinated to the oxygen. At the limit, this can produce a change in the coordination geometry around those metallic atoms, if they are capable of existing in more than one kind of coordination. In such cases one can no longer speak of anion vacancies in the usual sense of point defects since they are elements of the structure which are just unoccupied<sup>9</sup>. These subtle changes in coordination are difficult to detect unless they are ordered<sup>10</sup> and it is not uncommon in the anion-deficient perovskite for an ordered

Fig. 3 Electron diffraction pattern of  $\text{SrTiO}_{2.8}$  showing two sixfold superlattices along  $^*111$  and  $^*1\bar{1}\bar{1}$  of the perovskite subcell. Zone axis  $[110]$ .



arrangement of vacancies<sup>11</sup> to be found. This is the case for  $(\text{Ba}, \text{Sr})\text{MnO}_{3-x}$  (ref. 12) and  $\text{BaFeO}_{3-x}$  (ref. 13) where the  $\text{Mn}^{3+}$  and  $\text{Fe}^{3+}$  ions are ordered having bipyramidal-trigonal and tetrahedral coordination respectively. But, as  $\text{Ti}^{3+}$  is not a typical ion, adopting coordination geometries other than octahedral, we cannot yet give a structural model to explain this superperiodicity. However, the evidence shown above strongly suggests that in the synthesis conditions that we used, anion deficiency in  $\text{SrTiO}_{3-x}$  can be accommodated by planar defects parallel to  $(111)$

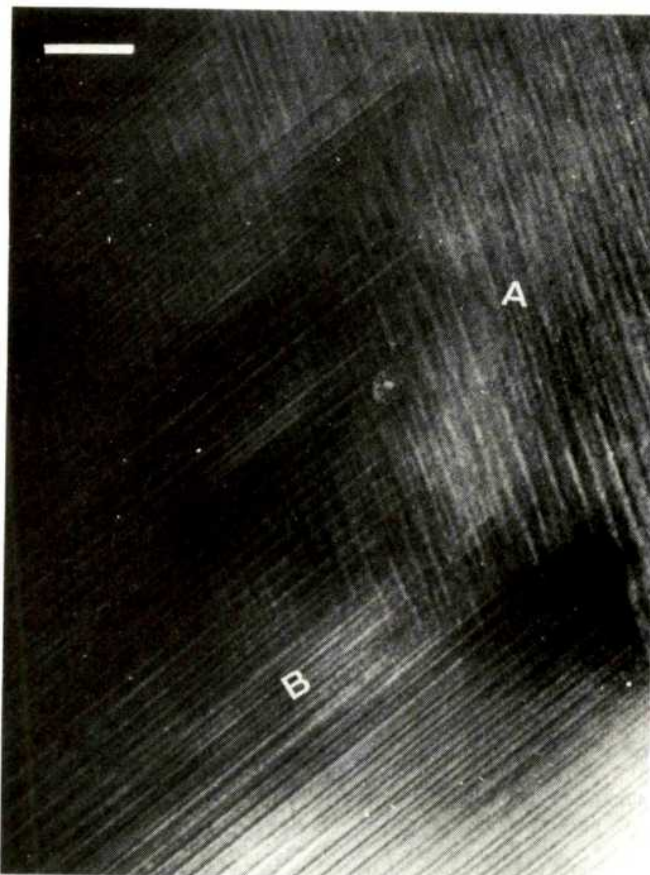


Fig. 4 Electron micrograph corresponding to Fig. 3. Note that the superlattice fringes intersect without apparent distortion and that many of them, especially those of set B, end within the crystal. Scale bar, 50 Å.

of the cubic subcell. In other words, there seems to be some kind of long-range order of the empty anion positions. It can also be expected that with different separations between these planar defects a family of new ordered phases, based on the perovskite structure, can be obtained. Furthermore, since we have commonly found two sets of crossing extended defects, it could well be that a new kind of block-type structures, analogous to those formed by double crystallographic shear on  $\text{ReO}_3$ -type oxides, can be formed based on the perovskite subcell. Experiments are now in progress to test this possibility and also to determine to what extent planar defects affect the properties of strontium titanate.

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1. Kestigian, M. Dickinson, J. G. & Ward, R. J. *Am. chem. Soc.* **5**, 5598-5601 (1957).
2. Goodenough, J. B. & Longo, J. M. *Crystallographic and Magnetic Properties of Perovskite and Perovskite Related Compounds*. Landolt, Börnstein New Series, Group III, Vol. 4a. New York: Springer Verlag (1970) p 126-314.



3. Cardona, M. *Phys. Rev.* **140**, A651-A655 (1965).
4. Lee, C. Y. & J. L. Brebner. *J. L. Phys. Rev.* **B3**, 2525-2533 (1971).
5. Koonce, C. S., Cohen, M. L., Schooley, J. E., Hosler, W. R. & Pfeiffer, E. R. *Phys. Rev.* **163**, 380-390 (1967).
6. Chang, T. S., Holzkichter, J. S., Jmbusch, G. F. & Schawlow, A. L. *Solid state Commun.* **8**, 1179-1181 (1970).
7. Anderson, J. S. in *Surface and Defect Properties of Solids* (eds Roberts, M. W. & Thomas, J. M.) **1**, 1-53 (Chemical Society, London, 1972).
8. Yamada, H. & Miller, G. R. *J. Solid State Chem.* **6**, 169-177 (1973).
9. Anderson, J. S. in *Modern Aspects of Solid State Chemistry* (ed. Rao, C. N. R.) 29-106 (Plenum, New York, 1970).
10. Gorter, E. W. *Proc. Intern. Congr. Pure and Appl. Chem.* 17th Congr. **1**, 300-306 (1959).
11. Negas, T. & Roth, R. S. *Proc. 5th Materials Research Symp. Solid State Chem. N.B.S. Spec. Publ.* **364**, 233-263 (1972).
12. Jacobson, A. J. & Horroxx, A. J. W. *Acta Crystallogr.* **B32**, 1003-1008 (1976).
13. Jacobson, A. J. *Acta Crystallogr.* **B32**, 1087-1090 (1976).

## Climatic information from $^{18}\text{O}/^{16}\text{O}$ analysis of cellulose, lignin and whole wood from tree rings

MEASUREMENTS of stable isotope ratios on tree rings have proved a promising way of determining past climate<sup>1-5</sup>. Recent discussions have centred on which component of wood provides the most reliable record<sup>6,7</sup>. We have measured the oxygen isotopic composition of cellulose, whole wood and lignin from tree rings in a white spruce (*Picea glauca*) which grew in the Edmonton area from 1882 to 1969. Using meteorological records we have evaluated the responses of the  $^{18}\text{O}/^{16}\text{O}$  ratios of the three components of the tree rings to seasonal temperatures. Results show a high correlation between cellulose  $^{18}\text{O}/^{16}\text{O}$  ratios and mean annual temperature, a poorer, though still significant correlation between whole wood  $^{18}\text{O}/^{16}\text{O}$  ratios and mean annual temperature and no significant correlation between lignin  $^{18}\text{O}/^{16}\text{O}$  ratios and mean annual temperature.

Whole wood measurements were made on 5-yr groups of rings taken from a 1-cm wide section across the tree. The wood was ground to pass through a 60  $\mu\text{m}$  mesh sieve and solvent extracted first in a benzene-methanol mixture and then in acetone to remove lipids tars and resins. The remaining material was then dried in a vacuum oven for 3 d. Combustions were carried out in an evacuated nickel reaction vessel<sup>1,8</sup>.  $^{18}\text{O}/^{16}\text{O}$  ratios were measured on a 90° magnetic sector isotope ratio mass spectrometer and are expressed as a  $\delta$  value ‰ with respect to standard mean ocean water (SMOW)<sup>9</sup>

$$(\delta^{18}\text{O}_x = [(^{18}\text{O}/^{16}\text{O})_x / (^{18}\text{O}/^{16}\text{O})_{\text{SMOW}} - 1] \times 1,000\text{‰}).$$

Reproducibility of the whole wood analyses (and that of the cellulose analyses) was  $\pm 0.2\text{‰}$ , the same as for the mass spectrometer.

Cellulose was extracted from each of the 5-yr groups of rings using the method of Green<sup>10</sup> which allowed the quantitative determination of  $\alpha$ -cellulose, hemicellulose and lignin in each sample. (Care was taken to ensure standardisation of procedure for all samples.) Isotope ratios of solvent-extracted wood were found to be the same within experimental error as those of dry whole wood. Also, the isotopic compositions of hemicellulose and  $\alpha$ -cellulose were found to be identical. This allowed the isotopic composition of the remainder (mostly lignin) to be calculated. Combination of the errors in the isotopic measurements of cellulose and whole wood, together with errors in measurements of percentage composition, yield an estimated error in the lignin isotopic composition of  $\pm 0.6\text{‰}$ . The results are given in Table 1.

The correlation coefficients of the isotope ratios of these components with seasonal temperature data were calculated. The results are shown in Table 2. For convenience the time period was divided into three-monthly groups such as January, February and March (Winter) and so on. The oxygen isotopic composition of cellulose is seen to have a significant correlation (95% confidence level) with mean seasonal temperatures throughout the year of growth. Correlation with mean annual temperature beginning with autumn previous to the calendar year of growth was found to be 0.94. The whole wood  $^{18}\text{O}/^{16}\text{O}$  measurements show significant correlation only with mean temperatures for winter and spring of the calendar year (0.60) yielding a correlation coefficient with

**Table 1** Oxygen isotopic compositions of cellulose, extracted whole wood and lignin

Sample	Cellulose $\delta^{18}\text{O}$ (‰)	Whole wood $\delta^{18}\text{O}$ (‰)	Lignin $\delta^{18}\text{O}$ (‰)	Lignin %	M.A.T.* (°C) (Sept-Aug)
1890-94	23.9	19.1	14.1	49.0	2.6
1895-99	23.4	19.6	15.4	47.4	2.3
1900-04	24.1	19.3	12.8	42.6	3.0
1905-09	24.7	19.9	15.2	50.5	3.1
1910-14	24.7	19.8	15.3	52.2	3.2
1915-19	24.1	19.6	15.1	49.8	2.7
1920-24	24.2	19.4	15.0	52.4	2.7
1925-29	23.6	19.1	14.5	49.3	2.3
1930-34	24.7	20.0	15.4	50.3	3.2
1935-39	22.9	18.3	13.2	47.2	2.1
1940-44	24.5	18.7	12.1	46.9	3.1
1945-49	23.3	17.7	11.2	46.2	2.5
1950-54	23.3	17.6	11.1	46.7	2.1
1955-59	24.5	19.4	13.8	47.6	3.1
1960-64	25.2	19.1	10.2	40.6	3.7
1965-68	24.0	18.7	12.5	46.0	2.4

Oxygen isotopic compositions ( $\delta^{18}\text{O}$  against SMOW) of cellulose, extracted whole wood and lignin for 5-yr groups of tree rings, together with the lignin concentrations (%).

\*5-yr mean annual temperatures calculated from September of the previous year to August of the final year.

mean annual temperature (September to August) of 0.55, significantly lower than that for cellulose. Correlation between  $^{18}\text{O}/^{16}\text{O}$  ratios of lignin and mean annual temperatures (September to August) is essentially zero ( $-0.03$ ). Also, there seems to be no relation between the amount of lignin present in the wood sample and seasonal temperature (Table 1). We conclude, therefore, that the lignin component of the wood carries no useful temperature information. The explanation for this may lie in the process of lignification of wood which takes place over a much longer period than that in which cellulose is produced and deposited in the tree ring<sup>11</sup>. It is concluded, therefore, that the only temperature information contained in  $^{18}\text{O}/^{16}\text{O}$  ratios of whole wood is due to that carried by the holocellulose component ( $\approx 50\%$ ).

The overall good response of the cellulose isotopic composition to mean seasonal temperatures may be explained in terms of the integrating effect of the tree ring growth. Measurements of the isotopic composition of tree ring material may supply information on the temperatures prevailing during the production of sugars which are subsequently converted into cellulose. This may not coincide with the actual period of ring growth. The production of early wood in the tree ring is a rapid process which may use material stored in the tree as photosynthesis occurred late in the previous year and, to a lesser extent, throughout the winter. Late wood production, on the other hand, is likely to use material produced during the growth season of the tree. Thus, isotopic analysis of cellulose from tree rings may well provide an excellent integrator of mean annual temperature including a period before growth actually occurs.

In conclusion, for the tree species and climate zone being discussed, it seems that only cellulose  $^{18}\text{O}/^{16}\text{O}$  ratios have a sufficiently good response with seasonal mean temperature to be used as a reliable indicator of past temperatures. Measurements of  $^{18}\text{O}/^{16}\text{O}$  ratios of whole wood, although much faster to carry out, involve less work in extraction procedures and avoid possible contamination from chemical reagents, seem to yield much less reliable temperature information. Lignin, because of the difficulty

**Table 2** Correlation coefficients of  $^{18}\text{O}/^{16}\text{O}$  composition of wood fractions with mean seasonal and mean annual temperatures

	Autumn	Winter	Spring	Summer	Whole year
Cellulose	0.70*	0.80*	0.45*	0.42*	0.94*
Whole wood	0.30	0.52*	0.50*	-0.30	0.55*
Lignin	-0.10	0.10	0.12	-0.40*	-0.03

(Autumn = October, November and December and so on)

\*Significant correlation at the 95% confidence level.

of carrying out direct  $^{18}\text{O}/^{16}\text{O}$  measurements does not, at this time, present an alternative. We believe that this calibration procedure should be carried out for any species being considered for use as a climate indicator in a given climatic zone before the usefulness of cellulose or whole wood  $^{18}\text{O}/^{16}\text{O}$  ratios can be evaluated.

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1. Gray, J. & Thompson, P. *Nature* **262**, 481–482 (1976).
2. Epstein, S. & Yapp, C. J. *Earth planet. Sci. Lett.* **30**, 253–261 (1976).
3. Yapp, C. J. & Epstein, S. *Earth planet. Sci. Lett.* **34**, 333–350 (1977).
4. Libby, L. M. & Pandolfi, L. J. *Nature* **266**, 415–417 (1977).
5. Wilson, A. T. & Grinstead, M. J. *Nature* **257**, 387–388 (1975).
6. Epstein, S. & Yapp, C. *Nature* **266**, 477–478 (1977).
7. Libby, L. M. & Pandolfi, L. J. *Nature* **266**, 478–478 (1977).
8. Thompson, P. & Gray, J. *Int. J. Rad. Isotopes* **28**, 411–415 (1977).
9. Craig, H. *Science* **133**, 1833–1834 (1961).
10. Green, J. W. in *Methods in Carbohydrate Chemistry* 3 (ed. Whistler, R. L.) (Academic, London, 1963).
11. Fritts, H. C. *Tree Rings and Climate* (Academic, London, 1976).

## Chemical structure of humic substances

THE nature of soil humic acids and similar substances derivable from oxidised brown coal is of considerable fundamental and practical interest. It has now been found, using organic extractants, that solutions of soil humic substances, capable of NMR spectroscopic investigation, may be prepared. The spectra obtained suggest that polymers of a similar structure to oxidised polyethylene are important components of soils. Numerous other types of chemical structures are also evident from the NMR absorptions of soil organic matter solutions; the amounts and types present vary widely from soil to soil. It is suggested here that chemical fractionation of soil organic matter can be most easily followed by observation of the NMR spectra.

Previous studies of organic matter extracted from soils have mainly used degradative techniques (oxidative<sup>1,2</sup>, pyrolysis–gas–liquid chromatography–mass spectrometry<sup>3,4</sup>) or infrared spectroscopy<sup>5–10</sup>. There is no general agreement on the chemical nature of soil organic matter. Interpretation of the infrared spectra of soil extracts has been hampered by the presence of complexed inorganic substances. Humic acids undoubtedly exist as complex organo–mineral–cation aggregates in nature<sup>11</sup>, this probably contributing to their stability under soil conditions.

Attempts to separate the inorganic and organic part of soil humic substance molecules without undue disruption of the latter have not been particularly successful. The presence of inorganic contaminants undoubtedly obscures some of the fine details of the infrared spectra and profound band broadening effects may also be caused by the existence of 'acid salt' hydrogen bonding<sup>12</sup> in humic acids.

Extracts of humic materials obtained from soils by traditional aqueous alkaline solutions give  $^1\text{H}$ -NMR spectra showing colloidal and solvent HO groups only. Progressive deuteration of the desiccated aqueous extracts leads to some resolution of the H–C groups in the NMR spectra but, owing to the difficulty of completely removing HO groups which cause band broadening by the formation of colloidal aggregates, the spectra obtained are poor quality. Also, the preferential solvation of O groups on the periphery of the macromolecules in aqueous systems apparently tends to remove the H–C structures from the solution phase, such hydrophobic groups occurring in a solid phase in the interior of

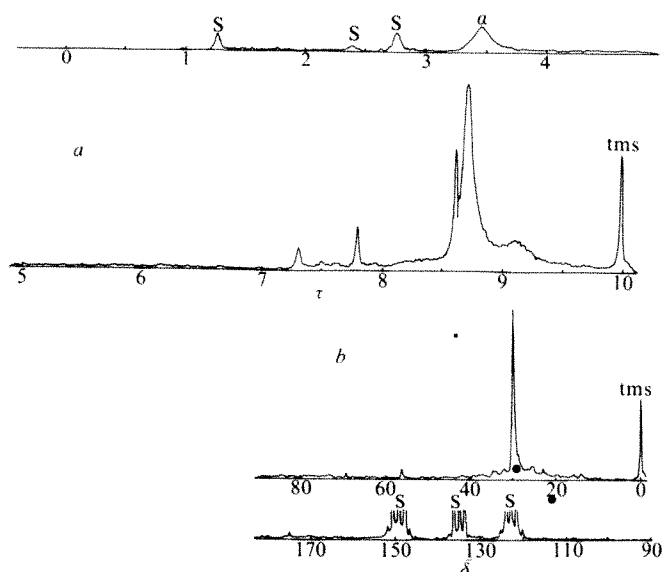
the macromolecules (which is NMR inactive under high resolution conditions).

$^1\text{H}$ -NMR absorptions found in deuterio-dimethylsulphoxide or deuterio-pyridine solutions are much more distinct than the spectra (obtained in  $\text{D}_2\text{O}$ ) of aqueous extracts and indicate the presence of  $\text{CH}_3(\text{CH}_2)_n$ , various environments of  $\text{CH}_2(\text{CO})$ ,  $\text{CH}_2\text{—NH—}$ , carbohydrate  $\text{H—C—O—}$ , and usually to a minor extent, some aromatic groups. In general, spin–spin coupling is rarely detected in soil organic matter extracts, this being attributed to the presence of a large number of similar structures with overlapping peaks. Unsaturated groups are generally absent at NMR-sensitive concentrations. The  $\text{CH}_3(\text{CH}_2)_n$  parts of the  $^1\text{H}$  spectra resemble those of some crude oil GPC fractions<sup>13</sup> but the  $^{13}\text{C}$  Fourier transform spectra are apparently less similar to such materials.

Fractions of several soils were obtained by extracting successively with: (1)  $(\text{CH}_3)_2\text{CO}$ ; (2)  $\text{HCOOH}$ ; (3)  $(4)^*(\text{CH}_3)_2\text{CO}$ ; (5)\*  $6\text{M HCl}$ ; (6)  $(\text{CH}_3)_2\text{CO}$ ; (7)  $\text{HCOOH}$ ; (8)  $(\text{CH}_3)_2\text{CO}$ , by Soxhlet extraction or \*cold extractions. This exhaustive extraction procedure removes essentially all the organic matter from the soil, and it can, therefore, be shown that polymethylene chains comprise up to ~30% of the total organic matter; however, fraction (1) may contain larger amounts of polymethylene chain components, as is demonstrated by the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra shown in Fig. 1 (a and b respectively) obtained from a  $(\text{CH}_3)_2\text{CO}$  Soxhlet extract of a podzol A horizon where 8% of the total organic matter was extracted. The  $^{13}\text{C}$  NMR spectrum resembles that of polyethylene, the principal absorption present being attributed to  $(\text{CH}_2)_n$ .

The proportion of  $(\text{CH}_2)_n\text{CH}_3$  present is best estimated from the  $^1\text{H}$  NMR spectrum, Fig. 1a (the  $^{13}\text{C}$  NMR spectrum was obtained under standard broad band  $^1\text{H}$  decoupling conditions where the otherwise quantitative peak area proportionality with the numbers of resonating nuclei is inexact). This shows that 72% of the H–C groups of the humic fraction occurs in  $(\text{CH}_2)_n\text{CH}_3$  structures, there being two superposed groups of peaks, one from (average)  $n = 7$  at  $\tau = 8.69$  ( $w_1^2$ , 6 Hz) and the other from  $n \approx 20$  at  $\tau = 8.7$  ( $w_1^2$ , ~60 Hz), the latter is suggested to be a fraction of semi-colloidal properties. This spectrum also indicates the presence of carbohydrate-like substances,  $\tau = 5.1\text{--}6.8$ , ~6% of H–C and a small (<2%) amount of H–C (aromatic) at  $\tau = 2.9$  and possibly also some (~3%) heteroaromatic groups at  $\tau = 1.2\text{--}2.0$ . The remaining H–C at  $\tau = 7.3\text{--}8.6$  is attributed to 'side chain' structures having H–C groups deshielded by O and N

Fig. 1 NMR Spectra of podzol humus,  $(\text{CH}_3)_2\text{CO}$  Soxhlet extract. Solvent: d-pyridine, tms internal standard; a,  $^1\text{H}$  60 MHz; b,  $^{13}\text{C}$ , FT, 20 MHz spectra; a,  $\tau$  value varies with dilution; s, solvent absorptions.



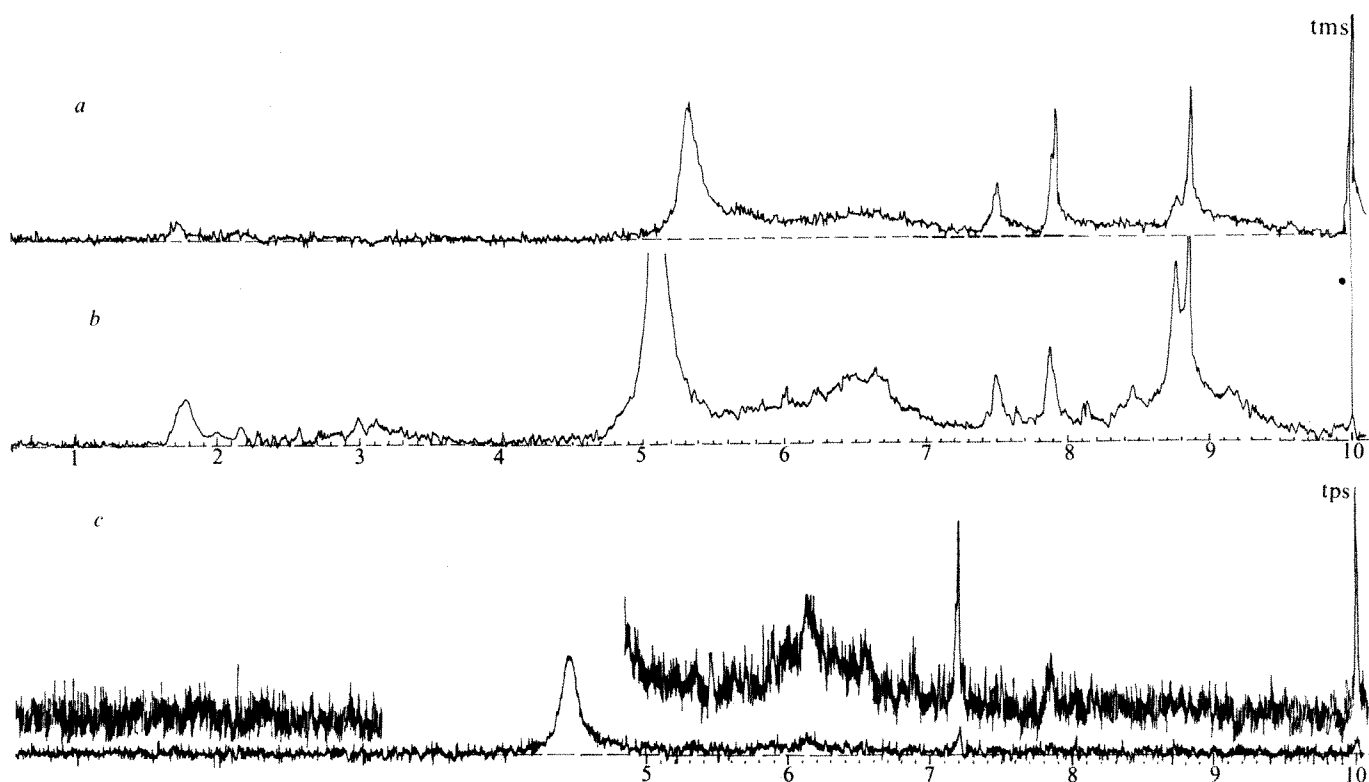


Fig. 2 NMR spectra of soil extracts, by HCOOH treatments. *a*, fraction (3) chernozem, in d-DMSO, tms internal standard; *b*, fraction (3) calcareous gley, in d-DMSO, tms internal standard; *c*,  $Zr^{4+}$  precipitated, deionised, HCOOH-DMSO (4:1) extract of Trawscoed E.H.F. 9-yr grazed ley; brown forest soil, in  $D_2O$ , tps [sodium, 3(trimethylsilyl)-1-propanesulphonate] internal standard.

atoms as well as by small (aliphatic) rings of C atoms. Some  $^1H$  NMR spectra of other organic matter fractions which are more typical of the main structural groups present in the firmer-bound fractions of soil organic matter, are shown in Fig. 2, where the amounts of  $(CH_2)_nCH_3$  groups of the total H-C present are: spectrum *a*, 24%, spectrum *b*, 31% and spectrum *c*, 1–2%.

Our results agree with the relatively few literature reports of the use of NMR for humic acid structure determination<sup>14–20</sup>, in particular the widespread occurrence of polymethylene-chain structures in humic acids is confirmed although the significance of this has not been previously noted. Spectra previously reported<sup>14,15,17</sup> of a 'fulvic' acid extracted from a podzol B horizon<sup>14,15</sup> and of humic acids extracted from lacustrine sediments<sup>17</sup> are dominated by the absorptions of polymethylene chains and are similar to the spectrum shown in Fig. 1*a*.

The long-term stability of polymethylene structures, such as those present in high molecular weight synthetic polyethylenes, is well known and the persistence of such structures in the soil, in humic acid molecules and related substances, is likely to be due to the resistance of the higher molecular weight fractions of polymethylene-based polymers towards biodegradation.

Another form of non-carbohydrate-type of structure that occurs in the humic fractions of soil is apparently dominated by polyketide structures with various relatively short aliphatic chains joined to C=O groups. Such molecules, as constituents of humic materials, are also indicated from the published NMR spectra of organic matter obtained from river water<sup>21</sup>, where they probably occur as metal ion complexes. Part of the fraction (6) in the above scheme has been found to consist of such structures, the NMR spectra becoming evident upon the removal of complexed Fe(III) by treatment with  $Na_4P_2O_7$  pellets in deutero-dimethylsulphoxide medium.

A search of the literature for evidence in support of the NMR results from other techniques indicated that a microbial 'humic acid' prepared from *Aspergillus flavus* culture had an infrared spectrum suggesting the presence of polymethylene chains<sup>22,23</sup>, indeed, a close similarity was indicated between this spectrum and

a published spectrum<sup>24,25</sup> of oxidised branched polyethylene. This microbial humic acid had a very low ash content (< 0.03%) and showed evidence of crystallinity. These are unusual features for substances which may be defined as humic acids; however, no soil humic acid has yet been prepared having such a low ash content and such a well resolved band structure in the infrared spectrum.

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1. Ogner, G. & Gjessing, E. T. *Geoderma* **14**, 139–145 (1975).
2. Schnitzer, M. & Neyroud, J. A. *Soil Biol. Biochem.* **7**, 365–371 (1975).
3. Bracwell, J. M. & Robertson, G. W. *J. Soil Sci.* **24**, 421–428 (1973).
4. Nagar, B. R. *Nature* **199**, 1213–1214 (1963).
5. Schnitzer, M. & Skinner, S. I. M. *Isotop. Radiat. Soil Org. Matter Stud. Proc. Symp. 2nd Int.* 41–55 (1968).
6. Otsuki, A. & Hanya, T. *Nature* **212**, 1462–1463 (1966).
7. Boldyrev, A. I., Matyushenko, B. T. & Polyakov, A. A. *Pochvovedenie* 81–88 (1969) *Soviet Soil Sci.* 222–228 (1969).
8. Thompson, S. O. & Chesters, G. *J. Soil Sci.* **21**, 265–272 (1970).
9. Shurukhina, S. I., Shurukhin, V. V. & Tarlakov, Yu. P. *Pochvovedenie* 146–149 (1973) *Soviet Soil Sci.* 240–243 (1973).
10. Bailey, J. R. *Plant and Soil* **40**, 285–302 (1974).
11. Bremner, J. M. *J. Soil Sci.* **5**, 214–232 (1954).
12. Shrivastava, H. N. & Speakman, J. C. *J. chem. Soc.* 1151–1163 (1961).
13. Coleman, H. J., Hirsch, D. E. & Dooley, J. E. *Analyt. Chem.* **41**, 800–804 (1969).
14. Barton, D. H. R. & Schnitzer, M. *Nature* **198**, 417–418 (1963).
15. Schnitzer, M. & Skinner, S. I. M. *Isotop. Radiat. Soil Org. Matter Stud. Proc. Symp. 2nd Int.* 41–55 (1968).
16. Oka, H., Sasaki, M., Itoh, M. & Suzuki, A. *Nenryo Kyokai-shi* **48**, 295–302 (1969).
17. Ishiwatari, R. *Chem. Geol.* **12**, 113–126 (1973).
18. Lüdemann, H.-D., Lentz, H. & Ziehm, W. *Erdöl und Kohle-Petrochemie-Brennstoff Chemie* **26**, 506–509 (1973).
19. Gonzalez, Vila, F. J., Lentz, H. & Lüdemann, H. D. *Biochem. biophys. Res. Commun.* **72**, 1063–1070 (1976).
20. Sciacovelli, O., Senesi, N., Solinas, V. & Testini, C. *Soil Biol. Biochem.* **9**, 287–293 (1977).
21. Reuter, J. H. & Perdue, E. M. *U.S. natn. Tech. Inform. Serv. Rep.* PB 210 714 (1972).
22. Visser, S. A. W. *Afr. J. Biol. appl. Chem.* **13**, 3–13 (1970).
23. Visser, S. A. & Mendel, H. *Soil Biol. Biochem.* **3**, 259–265 (1971).
24. Raff, R. A. V. *Ethylene and its Industrial Derivatives* (ed. Miller, S. A.) 377 (Benn, London, 1969).
25. Brandrup, J. & Immergut, E. H. *Polymer Handbook* **7**, 48–51 (Interscience, New York, 1966).



## Origin of stanols in young lacustrine sediments

STEROIDAL skeletons have been widely used as markers of the biological origin of organic material in ancient sediments<sup>1-7</sup> and petroleum<sup>8</sup>. Nevertheless, the origin and fate of sterols (stanols and stenols) in recent sediments are poorly understood. Here the significant contribution of organism-derived stanols (saturated sterols) to lacustrine sediments is reported and the geochemical significance discussed. The presence of stanols in recent and ancient sediments, together with unaltered sterols (unsaturated sterols) commonly found in algae, has been reported<sup>9-12</sup>. In view of the rare abundance of stanols in living organisms, this occurrence has been used as evidence that partial reduction of naturally occurring sterols had taken place over geological time. Stanols have also been identified in contemporary lacustrine sediments<sup>13-17</sup>. Based on the conversion of <sup>14</sup>C-cholesterol into <sup>14</sup>C-cholestanol under *in situ* incubation, Gaskell and Eglinton<sup>17,18</sup> proposed that such stanols in young sediments originate from sterols by microbiological reduction after deposition.

More recently, the same stanols found in contemporary lacustrine sediments were identified in phytoplankton, zooplankton<sup>15,19,20</sup> and various higher plants<sup>21</sup>, although in very small amounts (two or three orders of magnitude less than the total amount of sterol). In various surface soils, however, (0-3 cm in depth) and in the oxidising layer (0-1 cm in depth) of Suwa surface sediments, significant quantities of stanols were found<sup>21</sup>. Of the total sterol in the sediment samples, ~ 20% was composed of stanols. From these findings and from the difference in the degradation rates for stanols and sterols observed in incubation experiments, Nishimura and Koyama<sup>19</sup> concluded that stanols from organisms were concentrated, particularly under oxidative conditions, by the preferential degradation of sterols.

These results suggested that stanols in various sediments were derived not only from the sterol hydrogenation during sedimentation, but also directly from various living organisms. It is difficult, however, to estimate precisely the respective contribution in lacustrine sediments from the organisms and from the hydrogenation. The examination of a contemporary sediment in which virtually no sterol hydrogenation takes place would provide information on the contribution of organism-derived stanols to contemporary lacustrine sediments. The sediment is from Lake Shirakome-ike, Nagano, Japan (maximum depth, 8.6 m; area, 0.1 km<sup>2</sup>) which is an oligotrophic lake containing humic substances located about 2,100 m above sea level in the Yatsugatake mountain chain. Most of the sedimentary organic material may, therefore, be derived from terrigenous sources. The bottom water is oxygenated all year round. The sediment temperature ranges from 2 to 7 °C throughout the year.

Hayashi<sup>22</sup> investigated the microbial activity at four levels of the surface sediments (0-12 cm in depth). He found that the population ( $10^4$ - $10^5$  per g dry mud) of heterotrophic bacteria in the sediment layers was two orders of magnitude less than in Lake Suwa sediments in which sterol hydrogenation took place<sup>19</sup>, and that a greater proportion of the bacteria were spore-former types. On the other hand, the concentration of dissolved oxygen in the water during the stagnation period (November-July) virtually did not decrease with depth<sup>23</sup> and the consumption rate ( $9 \text{ mg O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) of  $\text{O}_2$  in the surface sediments was very slow relative to other lacustrine sediments<sup>22</sup>. These findings indicate that the microbiological activity in the surface sediments is extremely low.

Sediment samples were taken from the same core used by Hayashi<sup>22</sup>. The sample was collected from the deepest part of the lake in summer and was divided into sections at 3 cm intervals. A plankton sample was also collected with a plankton net in summer (the bloom season). Most of the plankton species were zooplankton, with the main species *Acanthodiatomus* sp. (~ 60%) and *Daphnia* sp. (~ 20%)<sup>24</sup>. All samples were frozen immediately and remained frozen until analysed.

The lipid extraction and the procedure for sterol analysis were as described by Nishimura and Koyama<sup>19</sup>. Structural identification of the sterols, following derivatisation to trimethylsilyl (TMS) ethers, was based on gas-liquid chromatography with co-injection of the authentic sterols on two types of columns and comparison of the mass spectra with those of authentic sterol TMS ethers<sup>13,25</sup>. The stereochemical assignment of the alkyl group at C-24 in C<sub>28</sub> and C<sub>29</sub>-sterol was not determined.

No interconversion of sterols to stanols was detected in control experiments employing the same procedures. The concentrations of total organic carbon, nitrogen and lipid carbon were obtained on a CHN analyser (Yanagimoto Model MT-1S).

Sterols (stanols and stenols) characteristically were distributed in some plankton species abundant in fresh-water lake and terrigenous sources, respectively; cholesterol and 5 $\alpha$ -cholestan-3 $\beta$ -ol (C<sub>27</sub>-sterols) are predominant in the plankton examined<sup>19</sup> and 24-ethyl-cholesterol and 24-ethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol (C<sub>29</sub>-sterols) in various plants and soils<sup>21</sup>. Therefore, the sterols in lacustrine sediments provide a useful indicator for estimating the autochthonous vs terrestrial contribution to the sedimentary organic matter. Table 1 summarises the sterol constituents identified in Shirakoma sediments and in zooplankton abundant in the lake. The major components in the sediments were 24-ethylcholesterol and 24-ethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol, while in the plankton cholesterol and 5 $\alpha$ -cholestan-3 $\beta$ -ol predominated. Moreover, the carbon number (C<sub>27</sub>, C<sub>28</sub> and C<sub>29</sub>) distribution of sterols in the sediments was very similar to those of *Larix* sp., abundant in the forest around the lake and in soil samples<sup>21</sup>. These facts indicate that most of the organic matter in the surface sediments was terrestrial in origin.

Total organic carbon, nitrogen, C/N ratio and lipid carbon in the surface sediments (0-20 cm in depth) are summarised in

**Table 1** The major sterol components of the sediments and plankton from Lake-Shirakoma-ike

Sterol	0-3		Core sample (depth in cm)				12-20		Plankton
	( $\mu\text{g}$ )*	(%)†	3-6	(%)	9-12	(%)	( $\mu\text{g}$ )	(%)	
Cholesterol	8.4	12	8.0	11	5.3	13	6.4	9	95
24-methylcholest-5,22-diene-3 $\beta$ -ol	trace	—	6.6	9	trace	—	2.1	3	ND‡
24-methylcholesterol	8.4	12	10.3	14	4.1	10	8.6	12	5
24-ethylcholest-5,22-diene-3 $\beta$ -ol	7.7	11	8.8	12	4.1	10	7.1	10	trace
24-ethylcholesterol	45.8	65	39.7	54	27.6	67	47.3	66	trace
Stanol									
5 $\alpha$ -cholestan-3 $\beta$ -ol	6.9	12	4.5	13	3.2	8	4.2	9	76
24-methyl-5 $\alpha$ -cholestan-3 $\beta$ -ol	5.4	9	3.8	11	4.1	10	4.6	10	8
24-ethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol	43.6	77	26.6	76	33.7	82	37.8	81	16
Total stanol	70.3		73.9		41.1		71.5		
Total stanol	55.9		34.9		41.0		46.6		
Total stanol/total sterol		0.44		0.32		0.50		0.39	0.006

\* $\mu\text{g/g}$  dry sediment.

†The percentage of total stanol and total sterol.

‡ND, not detected.

**Table 2** The vertical distribution of total organic carbon (T.O.C.), nitrogen (T.O.N.), C/N ratio and lipid carbon (Lipid C) in Shirakoma sediments

Depth (cm)	T.O.C.	T.O.N. (mg C(or N) per g dry sediment)	Lipid C	C/N
0-3	242	16	8.0	15
3-6	240	14	8.1	17
9-12	258	16	8.1	16
12-20	265	16	8.1	16

Table 2. All of the organic parameters were fairly constant irrespective of the sediment depth. In addition, the ratio of amino acid-, amino sugar-, insoluble- and  $\text{NH}_4^+$ -N to total nitrogen was constant throughout the sediment depth<sup>22</sup>. These facts strongly indicate that the microbiological activity in the sediments is greatly suppressed. This suppression is presumably due to the depletion of metabolisable organic compounds in the sediments<sup>26</sup> along with the low temperature (2–7 °C) of the sediment, because most of the organic matter is terrigenous material, composed mainly of diagenetically formed, secondary reaction products, like humic substances and kerogen<sup>27</sup>. Stenol hydrogenation in sediments, however, is a process effected by bacterial activity<sup>17,19</sup>. Thus, the contribution of stanols by way of the stenol hydrogenation process to the sediments, if any, may be extremely small.

Large quantities of stanols were found in all the sediment levels examined (Table 1). Particularly at the surface level (0–3 cm in depth), 44% of total sterol was composed of stanols. The relative abundance of stanols in the remaining levels ranged from 32 to 50%. The range was larger than that (20–30%) found in Suwa surface sediments (0–10 cm in depth) where stenol hydrogenation took place<sup>19</sup>. The high relative amounts of stanol in Shirakoma sediments presumably resulted from the concentration of stanol derived from organisms by the preferential degradation of stanols under oxidative conditions, until incorporation into the bottom sediments.

The plankton mixture from the lake yielded a very small amount of stanols (two orders of magnitude less than the amount of stanols) (Table 1). The major stanol was 5 $\alpha$ -cholestan-3 $\beta$ -ol. In comparison, ~ 80% of total stanol in the sediments was 24-ethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol (Table 1). This stanol is a major component in terrigenous-derived material (land plants and soils)<sup>21</sup>. This indicates that stanols derived from organisms on land may contribute significantly to stanols found in lacustrine sediments, which contain major proportions of terrigenous organic materials. Therefore, the relative abundances of C<sub>27</sub>-sterol (plankton origin) and C<sub>29</sub>-sterol (terrestrial origin) in young sediments may provide a strong clue to the origin of stanols (from organisms and/or from microbiological hydrogenation of stanols).

Gaskell and Eglinton<sup>17</sup> reported that stanols in Rostherne Mere sediment (0–30 cm in depth) were derived from stenol hydrogenation during burial. But the striking predominance of C<sub>29</sub>-sterols (24-ethylcholesterol + 24-ethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol) in both 7–18 cm and 18–30 cm levels of Rostherne sediment indicates that most organic materials were derived from terrigenous sources. This, coupled with the low temperature of Rostherne sediment (6–10 °C), indicates that most of the stanols in both 7–18 cm and 18–30 cm levels might originate directly from organisms on land by the preferential degradation of stanols during transportation to the bottom sediments. This may also be the case with the stanols in the 24–26 cm and 30–36 cm levels of Suwa sediments<sup>21</sup>.

Thus, the possible significant contribution of organism-derived stanols to sediments is not necessarily evidence for the operation of microbiological and/or chemical reduction of stanols during preservation. It is hoped that the ability to discriminate between stanols derived from the hydrogenation process and from organisms will provide information essential for understanding the origin and fate of steroid compounds in sediments.

In view of the scarcity of stanols in living organisms (land plants and various plankton species)<sup>19,21</sup>, the relative abundance

of stanols from organisms in early sedimentation may be a useful indicator of the operation of diagenetic processes under oxidative conditions. For example, the ratio of stanol to sterol (stenol + stanol) in Shirakoma sediments, various soils and Suwa surface sediments may provide information on the degree of diagenetic alteration undergone by the organic materials in the same sediments under oxidative conditions.

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- Burlingame, A. L., Huang, P., Belsky, T. & Calvin, M. *Proc. natn. Acad. Sci. U.S.A.* **54**, 1406–1412 (1965).
- Anderson, P. C., Gardner, P. M., Whitehead, E. V., Anders, D. E. & Robinson, W. E. *Geochim. cosmochim. Acta* **33**, 1304–1306 (1969).
- Kimble, B. J. *et al.* *Geochim. cosmochim. Acta* **38**, 1165–1181 (1974).
- Henderson, W., Wollrab, V. & Eglinton, G. in *Advances in Organic Geochemistry* (eds Schenck, P. A. & Havenaar, I.) 181–208 (Pergamon, Oxford, 1968).
- Anders, D. E. & Robinson, W. E. *Geochim. cosmochim. Acta* **35**, 661–678 (1971).
- Gallegos, E. J. *Analyt. Chem.* **47**, 1523–1528 (1975).
- Mulheirn, L. J. & Ryback, G. *Nature* **256**, 301–302 (1975).
- Hills, I. R., Smith, G. W. & Whitehead, E. V. *J. Inst. Petrol.* **56**, 127–137 (1970).
- Attaway, D. & Parker, P. L. *Science* **169**, 674–675 (1970).
- Mattern, G., Albrecht, P. & Ourisson, G. *Chem. Commun.* 1570–1571 (1970).
- Steel, G. & Henderson, W. *Nature* **238**, 148–150 (1972).
- Ogura, K. & Hanya, T. *Proc. Japan. Acad.* **49**, 201–204 (1973).
- Henderson, W., Reed, W. E. & Steel, G. in *Advances in Organic Chemistry* (eds von Gaertner, H. R. & Wehner, H.) 335–352 (Pergamon, Oxford, 1971).
- Gaskell, S. J. & Eglinton, G. in *Advances in Organic Geochemistry* (eds Tissot, B. & Biener, F.) 963–976 (Editions Technip, Paris, 1974).
- Nishimura, M. & Koyama, T. *Chem. Geol.* **17**, 229–239 (1976).
- Lee, C., Gagosian, R. B. & Farrington, J. W. *Geochim. cosmochim. Acta* **41**, 985–992 (1977).
- Gaskell, S. J. & Eglinton, G. *Geochim. cosmochim. Acta* **40**, 1221–1228 (1976).
- Gaskell, S. J. & Eglinton, G. *Nature* **254**, 209–211 (1975).
- Nishimura, M. & Koyama, T. *Geochim. cosmochim. Acta* **41**, 379–385 (1977).
- Isabell, C., Masuo, M. & Ikekawa, N. *Phytochemistry* **15**, 723–725 (1976).
- Nishimura, M. *Geochim. cosmochim. Acta* (in the press).
- Hayashi, H. *Jap. Soc. Ecology Abstr. A. Meet.* **23**, 23 (1976).
- Kurasawa, H. & Aoyama, K. *Miscellaneous Rep. Res. Inst. Natur. Resource* **63**, 9, 16 (1964).
- Okino, T. *Jap. Soc. Ecology Abstr. A. Meet.* **23**, 22 (1976).
- Brooks, C. J., Henderson, W. & Steel, G. *Biochim. biophys. Acta* **296**, 431–445 (1973).
- Sorokin, Y. I. *Microbiologia* **3**, 402–413 (1962).
- Welte, D. H. in *Advances in Organic Chemistry* (eds Eglinton, G. & Murphy, M. T. J.) 261–264 (Springer-Verlag, New York, 1969).

## Flandrian sealevel changes in the Thames Estuary and the implications for land subsidence in England and Wales

CATASTROPHIC flooding occurred in the Lower Thames and lowland coastal areas of eastern England in 1953. Large-scale investment in port, freight, factory facilities and connected urban development, has centred in these littoral and coastal zones. The effects of changing sealevels, such as seen in 1953, could, therefore, cause even greater economic and human loss in the future, unless government expenditure on flood alleviation schemes, as exemplified by the Thames barrage project, is increased and a coordinated flood protection policy established. Information on the rate of changing relative sealevel in south-east England is thus of great value. (A description is given here of a stratigraphic study upon the interleaved Flandrian biogenic and inorganic deposits of the Lower Thames estuary, carried out between central London and the Isle of Grain (Fig. 1).) From this, the heights of relative sealevel movements were determined and plotted against time to show the rate of relative sealevel change and subsidence trends for the Thames and southern England. The vegetational and environmental history was deduced from pollen, diatom and other micro-fossil analyses, with radiocarbon dating applied to establish an objective chronology.

The Flandrian sequences are developed on a fluvially-dissected late Devonian gravel surface, which falls progressively in height from west to east. The intercalated alluvial deposits continue this west-east dip, increasing in degree from

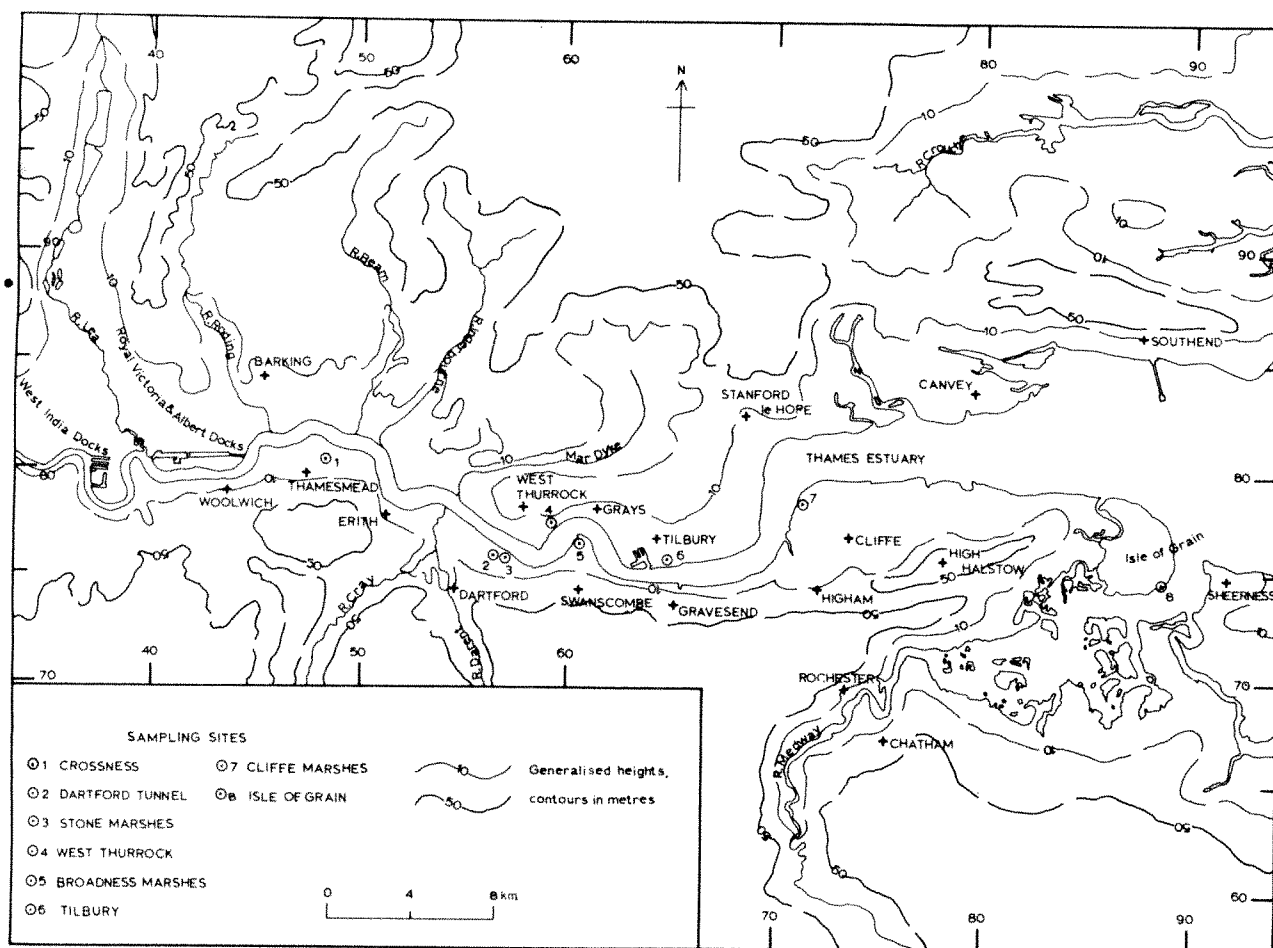


Fig. 1 Sketch map of the Lower Thames area.

Broadness Marsh eastwards. The inorganic fraction is composed of fine blue grey muds  $< 60 \mu\text{m}$  in diameter, with silt- and clay-sized particles dominant. From these phases of inorganic deposition five marine transgressions are recognised (see Table 1).

A complete profile of the diatom assemblages from Tilbury, in conjunction with other diatom and malacological studies, shows the importance of brackish water and tidal influences in the estuary from the outset in Thames I. The full marine effect was established soon after in the sequence, as characterised by the diatoms *Melosira sulcata*, *M. westii*, *Cymatosira belgica*, *Coscinodiscus excentricus* and *Raphoneis* spp. Towards the top of the deposits a fresh/brackish water influence becomes dominant. This shows a lessening of the marine effect nearer the present day, and is consistent with the accompanying decline in the rate of relative sealevel rise (Fig. 2).

The biogenic layers, formed by wood and monocotyledonous peats with gyttjas, show distinct internal changes in composition along an east-west transect. At the eastern end, *Phragmites* and saltmarsh peats dominate the deposits, changing upstream to form freshwater oak-alder fen wood peat. West of Broadness Marsh, all the sites showed seral vegetational development over the contact zones in the biogenic layers, changing from wood fen peat to *Phragmites* and then saltmarsh peat before the close and also before the onset of a marine transgression. This vegetational pattern supports the diatom evidence of an early and increasing salinity influence downstream and the rapid upstream progression of the tidal limit towards central London.

Pollen and macro-fossil analyses show the importance of the local influences of saltmarsh and fen environments on the vegetational history of the area. Arboreal pollen of *Alnus*, *Quercus*, *Corylus* type and *Tilia* occur in high frequencies

throughout. Following the recognition of the *Ulmus* decline at the Thames site at about 5,000 yr BP, non-arboreal pollen becomes dominant in the area's pollen rain, reflecting both increasing anthropogenic activity and rising sealevel. In a regional context, the rational limit of *Alnus* pollen<sup>1</sup> has been placed between  $8,510 \pm 110$  and  $8,170 \pm 100$  yr BP, with high *Alnus* frequencies occurring in a late Boreal VIc pollen context<sup>2</sup>. This dating is the earliest established at present for the rise of alder in south-east, central southern England and East Anglia. As such, it indicates the importance of wet valley conditions and related physiographic factors on the growth and expansion of this taxon in the Flandrian. From the biogenic deposits five regression phases, Tilbury I-V, have been recognised throughout the area, with Tilbury forming the type site.

From 19 radiocarbon-dated index points, taken from the regression and transgression contacts of the interleaved deposits, relative sea-level curves have been drawn for the area (Fig. 2). These contacts show the contemporary positions of MHWST, as determined by the biostratigraphic analyses. Compaction and consolidation corrections have not been used. Use of such corrections assumes a detailed geotechnical knowledge of the *in situ* behaviour of interleaved inorganic and biogenic deposits, which as yet has not been demonstrated<sup>3,4</sup>. Further, corrections from individual cores cannot be used by themselves to represent regional changes of level satisfactorily, because of the great local variability of the sediments. From Fig. 2 some general conclusions about the movement of sealevel can be drawn for the Lower Thames:

(1) 8,500/300–7,000 yr BP showed a rapid rise of sealevel from  $-25.5 \text{ m}$  to  $-8.9 \text{ m OD}$  (ordnance datum). Peat growth occurred on the basal Devonian sand and gravel surface, dependent on the rising freshwater table. No regression phases were recorded during this time. The rate of submerg-

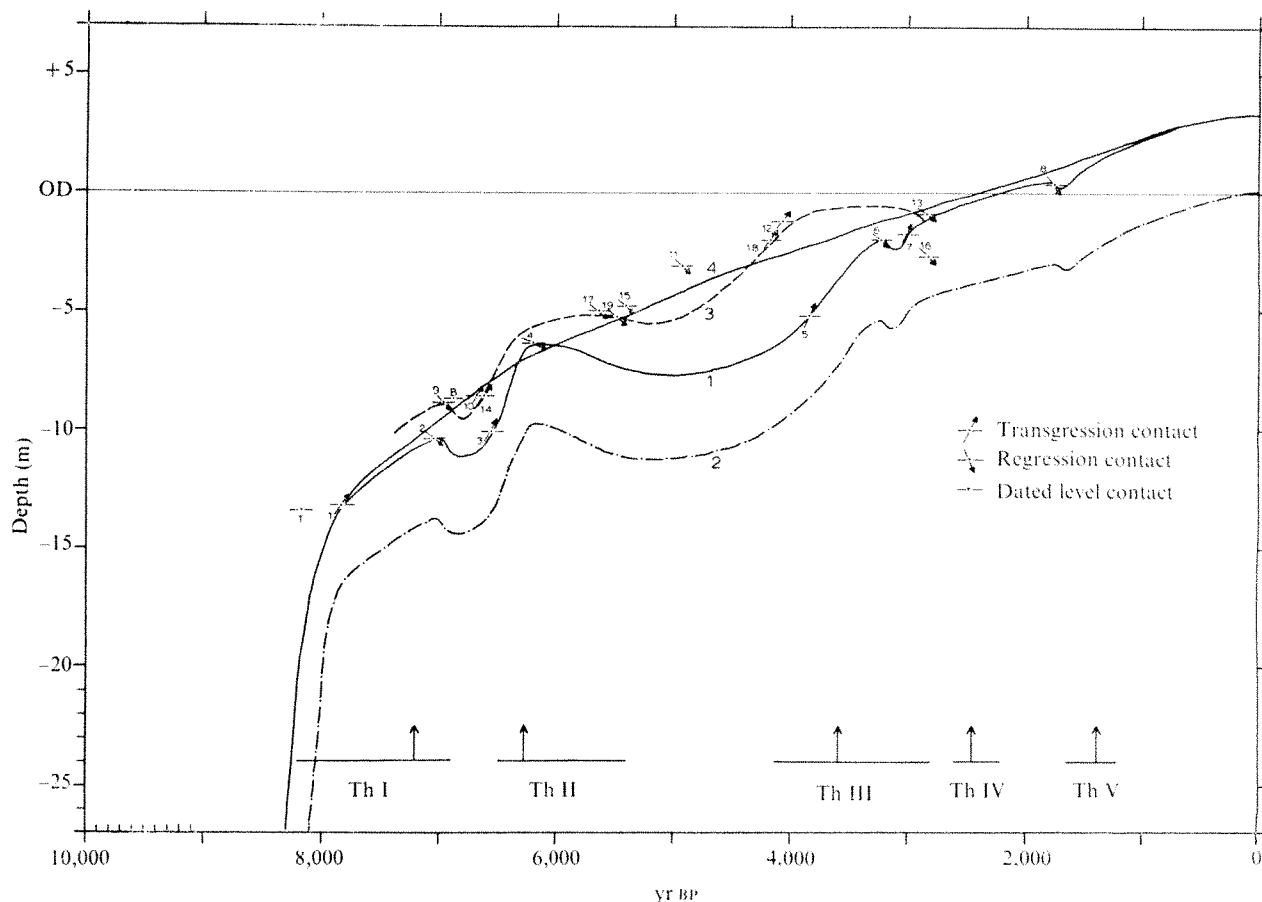


ence was approximately  $1.3 \text{ cm yr}^{-1}$ , compared with  $1.2 \text{ cm yr}^{-1}$  for the Netherlands<sup>5</sup> and  $1.5 \text{ cm yr}^{-1}$  for north-west England<sup>6</sup>.

(2) Sealevel fell for about 300 yr between 7,000 and 6,700 yr BP, with formation of alder wood peat.

(3) During Thames II sealevel rose between 6,600 and 5,500 yr BP from  $-10.1 \text{ m}$  to  $-5.0 \text{ m OD}$ . This forms the most extensive transgression recorded in the sequence, although the relative sealevel rise has fallen to  $0.5 \text{ cm yr}^{-1}$  compared to  $0.36 \text{ cm yr}^{-1}$  for the Netherlands.<sup>6</sup>

The 'best fit' line for the behaviour of sealevel, expressing the positive and negative movements represented by the intercalated clays and peats, is an oscillating curve (Fig. 2). A mean line for the index points, forming the relative sealevel curve 4, is also produced to show the overall trend of a steadily rising sealevel with time. These curves show a strong similarity in the form and rate of relative sealevel change with those of north-west Europe<sup>7</sup>. The timing and amplitude of movement in the Thames disagrees with the sealevel curve produced for the Essex coast<sup>8</sup>. This is probably because of the differing



**Fig. 2** Relative sea-level curves for the Thames Estuary. 1, Relative movement of mean high water spring tides (MHWST) at Tilbury. 2, Relative movement of mean tide level (MTL), derived from the difference between MHWST and MTL of the sample contact points. 3, Relative movement of MHWST from Crossness, Stone Marsh, Dartford Tunnel and Broadness. 4, Curve representing the mean line for the sampled contact points. Th I–V are transgression sequences recognised in the Thames estuary. Index points are as follows: 1–8, Tilbury (T); 9–13, Stone Marsh; 14–16, Broadness Marsh (B); 17–18, Crossness; 19, Dartford Tunnel. IG, Isle of Grain.

(4) Between 5,500/5,000 and 4,000 yr BP a major regression, Tilbury III, took place with formation of thick monocotyledonous peat, becoming wood fen peats upstream. Environmental conditions in the estuary would not have been sensitive enough to record more minor fluctuations in sealevel, shown for this time in the open coastal sediments of north-west England<sup>6</sup> (Fig. 3).

(5) Thames III shows a rapid rise in sealevel between 4,000 and 3,000 yr BP to levels between  $-1.4 \text{ m}$  and  $-2.5 \text{ m OD}$ . A minor regression phase Tilbury IV is recorded in the form of a thin silty peat. The timing and height of this event varies in the estuary and may result partly from different rates of sedimentation and relative subsidence in the estuary.

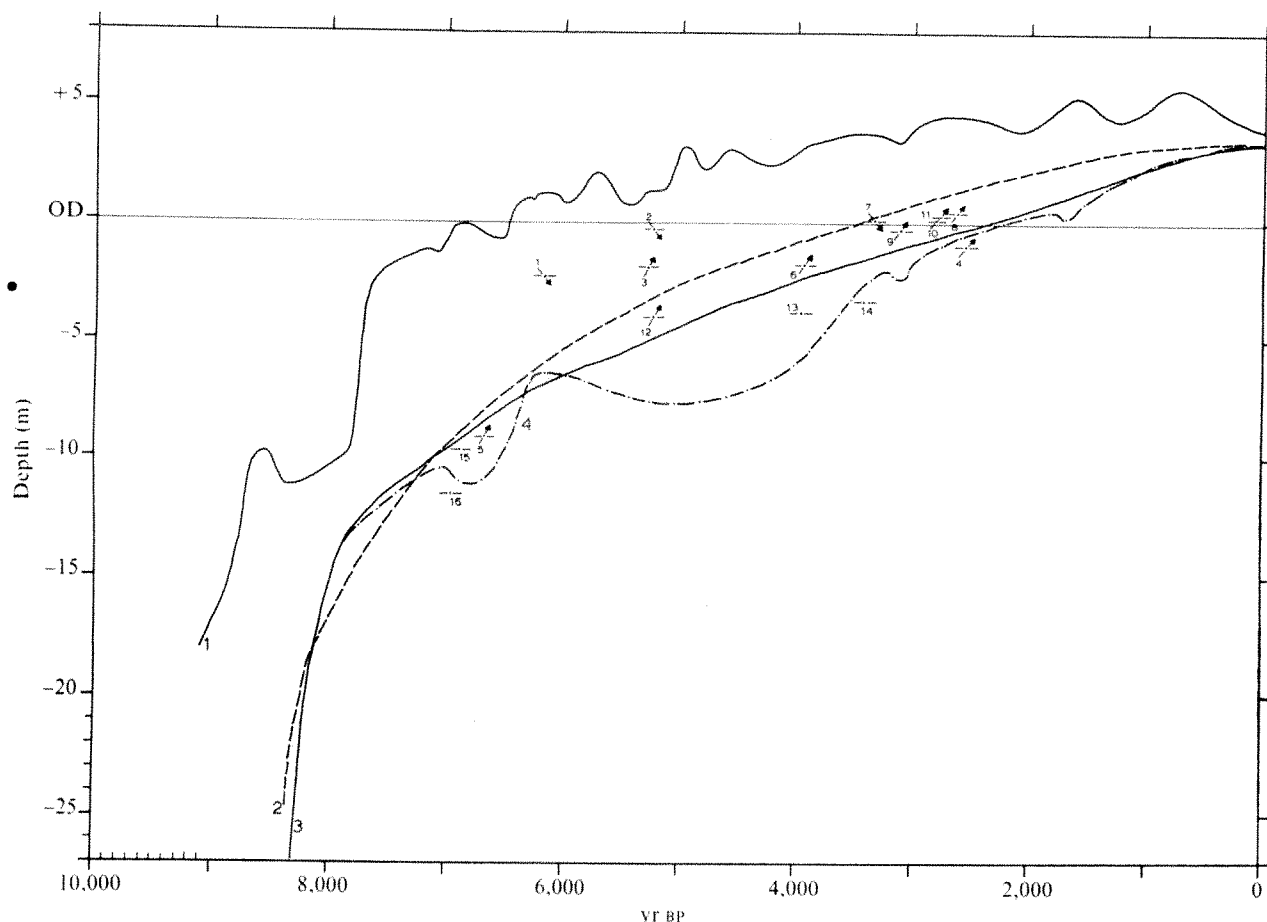
(6) Following this regression, sealevel rises above present ordnance datum (Newlyn), reaching  $+0.4 \text{ m OD} \sim 1,750 \text{ yr BP}$  at Tilbury. At this level, a thin non-persistent silty peat records a further regression termed Tilbury V.

(7) During Thames V, over the last 1,000 yr, sealevel for MHWST continued to rise rapidly, possibly reflecting increased embanking and building in the estuary.

environmental and sedimentological histories of the two areas, in addition to the wide variety of data sources used by Green-smith and Tucker for this area. Despite the local and regional inaccuracies implicit in sealevel data<sup>9,10</sup>, comparison of Flandrian relative sealevel curves from similar area-based studies in England and Wales has allowed trends in land subsidence to be drawn. Within the Thames, possible differential down-warping of about  $1.5 \text{ m}$  has been identified between Crossness and Tilbury for the Flandrian, shown by the differential between curves 1 and 3 (Fig. 2). Changes upstream in

**Table 1** Flandrian transgression sequences in the Lower Thames estuary

Thames V	..	..	..	..	..	$\sim 1,750 \text{ yr BP}$
Thames IV	..	..	..	..	..	2,600– / yr BP
Thames III	..	..	..	..	..	3,850–2,850 yr BP
Thames II	..	..	..	..	..	6,575–5,410 yr BP
Thames I	..	..	..	..	..	8,200–6,970 yr BP



**Fig. 3** Relative sealevel curves indicating subsidence trends in England and Wales. Transgression (upward arrow) and regression (downward arrow) contacts from intercalated peat and clay deposits in north-east and eastern England<sup>15</sup>. Relative sealevel curve 1 from north-west England<sup>6</sup>; curve 2 from south-west England (R.J.D. in preparation); curve 3 from the Lower Thames estuary (see Fig. 2); curve 4 represents the movement of MHWST at Tilbury, Lower Thames estuary (Fig. 2 and ref. 16).

the tidal amplitude in the estuary, however, may form an important component here. The regional trends of relative west-to-east and north-to-south down-warping in England<sup>11</sup> are supported (Fig. 3). By comparison, the amount of relative land subsidence for south-east England relative to the south-west, formerly given as 6.1 m since ~ 6,500 yr BP<sup>12</sup>, is not confirmed. This figure was based on inadequate field data and a lack of understanding of the inequalities in cross-continental correlations of sealevel movements—the changes in geoidal configuration, tectonic stability and tidal amplitude. Comparison of the curves derived from biostratigraphic and <sup>14</sup>C analyses of the transgression and regression contacts of intercalated deposits in these two areas, shows a consistent trend of down-warping of the south-east relative to the south-west. The value for this relative subsidence may be placed at 2–3 m for the Flandrian<sup>16</sup>. But, rate of down-warping are seen to vary with many local environmental parameters, for example, compaction and consolidation ratios, tidal regimes<sup>13</sup> and changes in the height of the regional sea surface<sup>10,14</sup>, making generalisation of subsidence values tenuous.

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1. Smith, A. G. & Pilcher, J. R. *New Phytol.* **72**, 903–914 (1973).
2. Godwin, H. *The History of the British Flora*. (Cambridge University Press, Cambridge, 1975).

3. Skempton, A. W. *Q. Jl geol. Soc. Lond.* **125**, 373–412 (1970).
4. Marsland, A. *Q. J. Engng Geol.* **10**, 1–26 (1977).
5. Jelgersma, S. *Meded. Geol. Sticht.* Ser. C **6**, 1–100 (1961).
6. Tooley, M. J. *Geol. J.* **11**, 137–152 (1976).
7. Morner, N.-A. *Palaeogeogr. Palaeoclimatol. Palaesecol.* **19**, 63–85 (1976).
8. Greensmith, J. T. & Tucker, E. V. *Geol. en Mijnb.* **52**, 193–202 (1973).
9. Tooley, M. J. *Geogr. J.* **140**, 18–42 (1974).
10. Morner, N.-A. *J. Geol.* **84**, 123–151 (1976).
11. Dunham, K. C. *Phil. Trans. R. Soc. Lond. A* **272**, 81–86 (1972).
12. Churchill, D. M. *Quaternaria* **7**, 239–249 (1965); *Proc. prehist. Soc.* **5**, 74–84 (1965).
13. Bowen, A. J. *Phil. Trans. R. Soc. Lond. A* **272**, 187–199 (1972).
14. Rossiter, J. R. *Geophys. J. R. astr. Soc.* **12**, 259–299 (1967).
15. Gaunt, G. D. & Tooley, M. J. *Bull. Inst. geol. Sci.* **48**, 25–41 (1974).
16. Devoy, R. J. thesis, Univ. Cambridge.

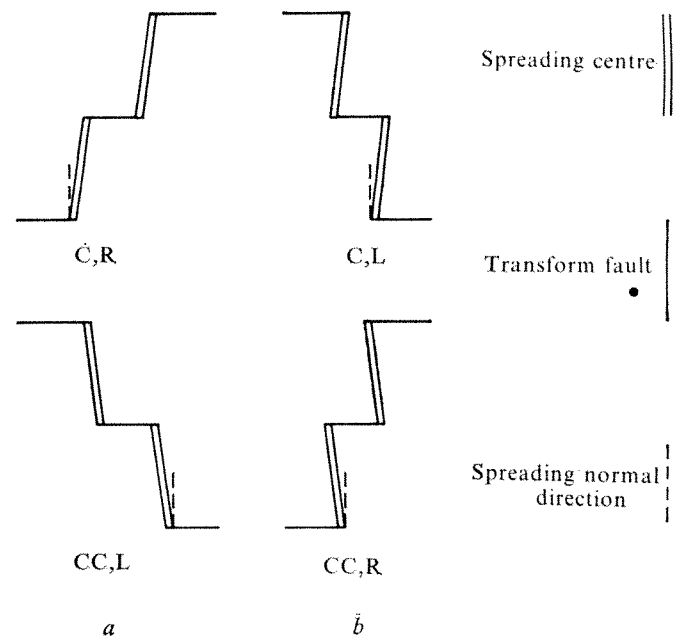
## Are spreading centres perpendicular to their transform faults?

A COMMON assumption in seafloor spreading is that mid-ocean ridge crests are aligned perpendicular to their transform faults and, hence, to their spreading directions. There are some well known exceptions to this rule, for example, the Reykjanes Ridge. Vogt *et al.*<sup>1</sup> suggested that spreading systems may take one of two configurations: either a transform faultless, oblique configuration or a perpendicular one. He then assigned the Reykjanes and certain older anomaly sets to the first category and the rest, the segmented, faulted ridges to the latter. We agree with this bimodal separation of ridge types, and here we discuss only the latter, the transform-faulted 'perpendicular' group. We examined all available detailed data from this group, and wherever we could

find a fine scale map which included both transform fault and spreading centre we measured their trends. Of eight segmented, slow-spreading centres (half-rate less than  $3 \text{ cm yr}^{-1}$ ) we did not find a case which was, in fact, perpendicular. All were  $6\text{--}38^\circ$  oblique, and all were oblique in the sense which shortens the connecting transform faults, that is, the configurations in Fig. 1a as opposed to those shown in Fig. 1b. Fast- and some intermediate-rate spreading centres, on the other hand, seem to be perpendicular within the errors of measurements. These results are particularly interesting for the constraints that they place upon models of spreading centres in which the ridge crest-transform fault angle is used as a measure of the relative amounts of energy dissipated by these two features as motion occurs across them.

While working on the masses of data recently collected in the famous area, we became convinced that the spreading centres are not perpendicular to the transform faults here. They are aligned  $17^\circ$  oblique to the spreading normal direction and their obliqueness is such that the transform faults are shortened (C, R in Fig. 1). Furthermore, this obliqueness has been quite stable through time. Before about 5 Myr ago, the spreading direction seems to have been about  $N 105^\circ E$  while the spreading centre trend was about  $N 35\text{--}45^\circ E$ , or about  $20\text{--}30^\circ$  oblique to the spreading normal. Between 5 and 3 Myr ago, the direction changed to E-W, causing the spreading centre to break into short segments and rotate to a  $N 17^\circ E$  trend. The small east-west transform faults in the Famous area formed during this change. This new oblique configuration seems to have continued to the present. The maintenance of the oblique relationship through the readjustment in directions shows that it is not a chance occurrence. It is the stable configuration for this spreading system.

Let us review the data which led to these conclusions. The alignment of the presently active transform faults and spreading centre are well documented. In the fracture zones, large, medium, and fine-scale topography as well as microearthquakes all show east-west lineations<sup>2-4</sup>. In the spreading centre, the central magnetic anomaly, the large-scale topography, and more than 700 fine-scale tectonic lineations (faults and fissures) mapped in the rift inner floor all have a pronounced  $N 17^\circ E$  strike<sup>5-9</sup> (Fig.



**Fig. 1** Spreading centre alignments which result in (a) shortening of the connecting transform faults and (b) lengthening of the transform faults. All measured oblique cases are the type shown in (a), that is transform shortening type (C, R or CC, L). C and CC signify clockwise and counter-clockwise rotations of the spreading centres with respect to the spreading normal directions. R and L signify right and left lateral offsets of the spreading centres by the transform faults.

2). There are a few north-south lineations in the inner floor, but they form part of a nearly symmetrical Gaussian distribution of lineation strikes which has a median of  $N 17^\circ E$  and a standard deviation of only  $6^\circ$  (ref. 5). Since the lineation pattern is normally distributed about  $N 17^\circ E$ , we feel that it is misleading to interpret

**Table 1**

Location	Angle of obliqueness	Sense of obliqueness*	~ Half spreading rate (cm yr <sup>-1</sup> )	Data type used†	Ref.
Mid-Atlantic Ridge					
11° N (Vema F. Z.)	$9^\circ \pm 1^\circ$	CC,L	1.5	T	14,15
15° N	$7^\circ \pm 5^\circ$	CC,L	1.5	T	16
30° N (Atlantis F. Z.)	$6^\circ \pm 4^\circ$	C,R	1	T	17
37° N (Famous Area)	$17^\circ \pm 3^\circ$	C,R	1	T,M,E	2,3,4,6
40° N (Kurchatov F. Z.)	$38^\circ \pm 5^\circ$	C,R	1	T	17,18
52° N (Charlie-Gibbs F. Z.)	$35^\circ \pm 5^\circ$	CC,L	1	T	19
Gulf of Aden					
48° E	$16^\circ \pm 6^\circ$	CC,L	1	T,M	20
51° E (Alula-Fartak F. Z.)	$18^\circ \pm 7^\circ$	CC,L	1	T,M	21
Juan de Fuca Ridge					
44° N (Blanco F. Z.)	$11^\circ \pm 3^\circ$	CC,L	3	M	22
Gulf of California					
23° N (Tamayo F. Z.)	$1^\circ \pm 3^\circ$	P	3	T,M	23,24
Galapagos Spreading Centre (Panama F. Z.)	$2^\circ \pm 3^\circ$	P	3	T,M,E	25
East Pacific Rise					
9° N (Siqueiros F. Z.)	$0^\circ \pm 4^\circ$	P	6	T	‡
3° S (Quebrada F. Z.)	$0^\circ \pm 3^\circ$	P	7.5	T	§
6° S northern	$4^\circ \pm 4^\circ$	P(C,R)	7.5	T,M	26
6° S southern	$4^\circ \pm 4^\circ$	P(CC,R)	7.5		26
N. E. Pacific					
40° N (Mendocino F. Z.)	$0^\circ \pm 2^\circ$	P	3.7	T,M	27,28
34° N (Murray F. Z.)	$-1^\circ \pm 3^\circ$	P	5.0	T,M	28,29

\*See Fig. 1 for explanation of symbols; P, perpendicular; F. Z., Fracture Zone.

†M, magnetic anomalies; T, topographic chart; E, earthquake epicentral locations.

‡B. R. Rosendahl and L. Dorman unpublished chart.

§P. F. Lonsdale, data from Pleiades cruise 1976.



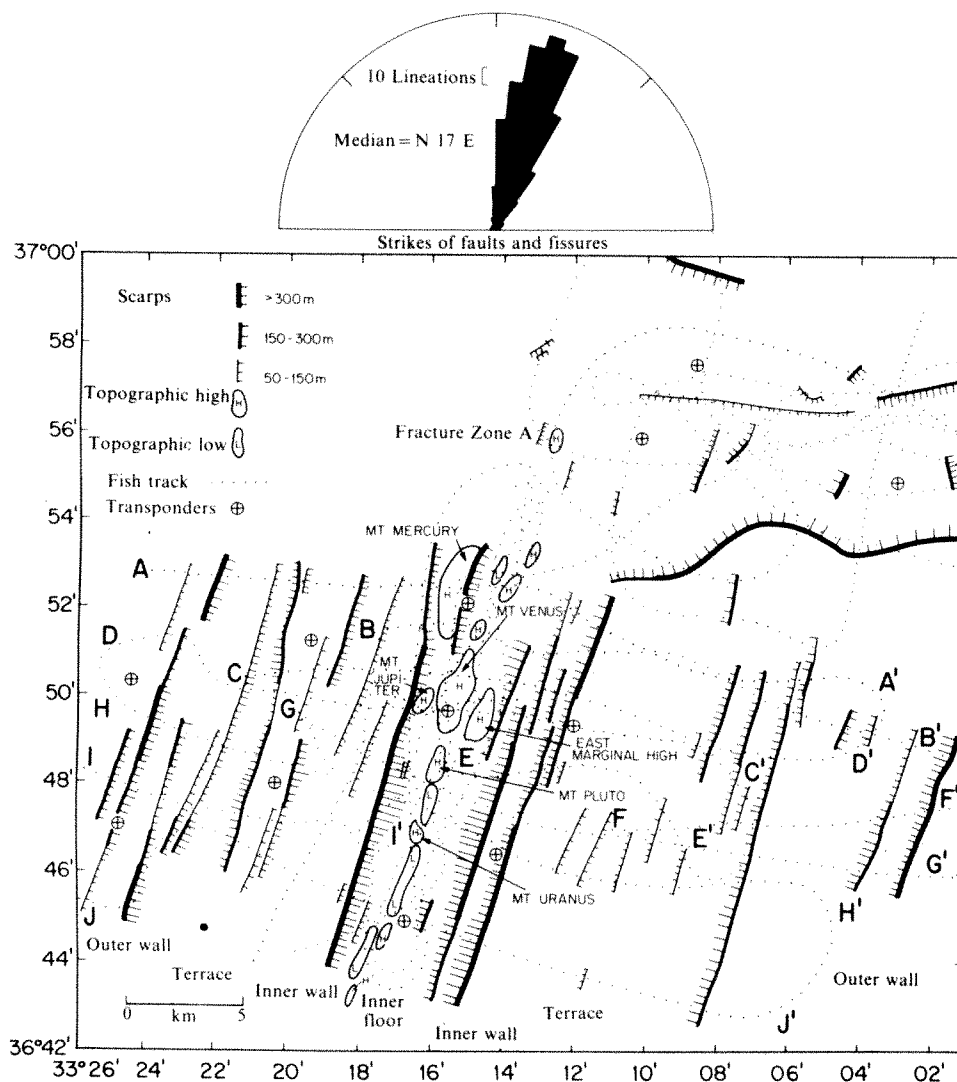
the observation of a few north-south lineations as evidence for readjustment to orthogonal spreading as some workers have done. The magnetic maximum along the inner floor axis is caused by crust less than 0.2 Myr old and it also has a N 17° E trend<sup>9</sup>. This is independent evidence that even the youngest crust in the inner floor is created in a direction oblique to the nearest transform faults.

Evidence for the alignment between recent times and 3 Myr, and for the preceding change in direction, comes mostly from the magnetic anomalies (Fig. 3), although their interpretation is supported by lineation of the large-scale ridge crest topography. Deep-tow and surface magnetic data indicate that magnetic anomalies back to 2' trend about N 17° E. Between anomalies 2' and 3' (2.5–5.2 Myr BP) the magnetic lineations rotate so that anomalies 3' and older show a trend of N 35–45° E. (Bird and Phillips<sup>10</sup> report a N 35° E average trend for the anomalies of 0–9 Myr age. It seems that the time interval they used in stacking the anomalies was too large to resolve the change in trend for anomalies less than 3 Myr old.) The magnetic anomalies in Fig. 3 are offset right-laterally back to anomaly 2'. Between anomalies 2' and 3' the anomalies change trend, the offsets decrease and the topographic fracture zones disappear. For anomaly 3' and older, the magnetic lineations are nearly continuous in a N 35–45° E direction. To establish the spreading direction before the change in direction and the creation of the Famous area fracture zones, we must find a larger, more continuous fracture zone. The nearest major one is the Oceanographer, at 35° N. There is some confusion about this feature since its overall trend is N105° E, not east-west like the smaller fracture zones. We interpret this to be

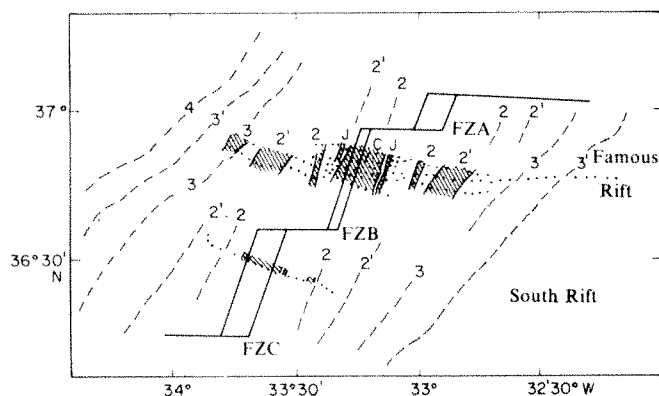
because the fossil direction, the direction of spreading before the change, and the present east-west motion has not continued long enough to develop a clear readjustment in the gross topography of this long offset fracture zone. Young east-west motion is shown by medium-scale east-west features around the zones<sup>11,12</sup> and by a tightly constrained focal mechanism solution<sup>13</sup>. Thus, we assume that the present direction of relative plate motion to be east-west while the direction before 5 Myr ago was 15° south of east. The older Famous area anomalies are 20–30° oblique to this direction.

Given this line of reasoning, we conclude the following: on the Mid-Atlantic Ridge at 35–37° N (1) spreading is stably oblique at present; (2) the spreading pattern has had a stable obliqueness of 17° in the present configuration for approximately 3 Myr; (3) spreading was 20–30° oblique in this area before 5 Myr and remained oblique through a change in spreading direction and creation of new fracture zones.

We may have made a mistake in using fracture zones A and B to delineate recent plate motions. In a re-evaluation of global plate tectonic solutions, T. Jordan and B. Minster (personal communication) and others have shown that the north-central Atlantic is one of several problem areas; in particular the east-west relative motion indicated by these transform faults is difficult to reconcile with other plate motions around the Azores triple junction. A more acceptable motion on those grounds would occur if the direction of relative plate motion in the Famous area were somewhat south of east. This implies an oblique opening across the Famous area fracture zones. While detailed deep-tow, microearthquake, and submersible studies<sup>2–4</sup> indicate that this is not the case, we cannot entirely rule it out. If



**Fig. 2** Tectonic map Famous area showing major scarps of rift valley and Fracture Zone A<sup>3,6</sup> and rose diagram showing orientation of 100 small lineations in the inner floor and walls<sup>4</sup>. Both large and small features show a N17° E trend for the rift valley. Distinct scarps show an approximate east-west trend for Fracture Zone A.



**Fig. 3** Sketch map of magnetic lineations in the Famous area<sup>9</sup> (modified from Phillips and Fleming, in preparation). Hachured areas show extent of magnetic anomalies as detailed by deep-tow survey<sup>9</sup>. Rift valleys and transform faults are shown as mapped from the bathymetry. Note that anomalies younger than 2' are aligned approximately N20° E and are offset by the transform faults while anomalies older than 2' are aligned approximately N40° E and are not significantly offset. The transform faults seem to have originated at the time of the change in direction, between anomalies 2 and 3.

Jordan's preferred direction is assumed, the amount of obliquity for the last 3 Myr is reduced (to 5–10°) but is not eliminated, and it is still in the transform shortening sense.

As we became convinced of the stable oblique spreading in the Famous area, we began to wonder if most 'perpendicular' spreading systems might be somewhat oblique. While no other area of the oceanic ridges is known as thoroughly as the Famous region, we found a number of sections which are well enough mapped either topographically, magnetically, or both to allow measurement of trends. For slowly spreading regions (half-rate less than 3 cm yr<sup>-1</sup>), we found eight such surveys which include both spreading centres and adjoining transform faults. These are listed in Table 1 with their angles of obliqueness and a subjective estimate of the uncertainty with which the mapped features constrain the trends. As shown all these ridges were found to be oblique, without exception, and, furthermore, every case was oblique in a transform-shortening sense (either C, R or CC, L). Not included in the table is Johnson and Vogt's<sup>31</sup> chart of the Mid-Atlantic Ridge 47–50° N. The 500-m contour interval of the chart was too coarse to make precise measurements. Most of the ridge segments, however, seem to be 15–40° oblique.

For fast spreading centres, only three high resolution, fine-scale maps exist which include both the ridge crest and the adjoining transform fault. These are shown in the Table. In all cases, the transform faults are perpendicular to the spreading centres.

In the survey at 6° S, the transform fault trend is controlled by only two crossings. The magnetic signature of the fault is so sharp, however, that we consider it to be reasonably controlled. Note that the southern intersection at 6° S has an obliquity of 4° in a transform lengthening sense, but within the measurement error, is indistinguishable from perpendicular. Rea<sup>26</sup> suggests that the odd trends in the area are due to a recent change in spreading direction with a lag in the topographic adjustment.

Some data exist in other fast spreading areas. Less detailed maps of the Pacific–Antarctic Rise indicate that the angle between the spreading centres and transform faults is never resolvable different from 90° (ref. 29). In the north-east Pacific, an older region of fast spreading has been quite well mapped. The configurations of the ancient ridge crests and transform faults are preserved in the magnetic lineations and fracture zones and these also are perpendicular, as shown in Table 1.

Reasonable surveys exist for three intermediate-rate spreading centres, half-spreading rate around 3 cm yr<sup>-1</sup>. As shown in Table 1, these give mixed results. The Juan de Fuca Ridge is unquestionably oblique, about 10° in a transform-shortening sense. The other two, the mouth of the Gulf of California and the

Galapagos Ridge, do not seem to be significantly different from perpendicular.

Why do spreading plate boundaries break up into alternating ridge and transform fault segments? Why don't they simply retain the irregular shape which they inherit from the original plate break up? Lachenbruch and Thompson<sup>32</sup> and Lachenbruch<sup>33</sup> suggest that the final stable configuration taken by the ridges and transform faults is the one which minimises the mechanical energy dissipated during the accretion and spreading processes. The perpendicular configuration is that which minimises the length of ridge crest in the system at the expense of adding whatever amount of transform fault is needed. Thus, if the minimum energy principle is correct, oblique configuration implies that the energies dissipated by the two features are more comparable. To quantify this comparison, Lachenbruch<sup>33</sup> developed a ratio,  $S/r_0$ , which is approximately equivalent to the ratio between the energy dissipated along the transform fault and that dissipated in the spreading centre. Configurations which are nearly perpendicular like those found at the fast spreading centres, result in a value for  $S/r_0$  near zero. In cases of oblique spreading the relationship between angles and energies is more difficult to evaluate. While transform faults are relatively well understood, the energy dissipated by spreading centres depends on the emplacement model assumed and very few constraints exist for such models. Lachenbruch<sup>33</sup> derives some appropriate equations describing the various likely model types. Using his models and our measured obliquenesses, the  $S/r_0$  ratio ranges from 0.2 to 1.6. In other words, the amounts of energy being dissipated by the transform faults and by the ridges in slow-spreading systems seem to be of about the same magnitude.

The concentration of earthquakes at transform faults shows that they are active dissipators of energy. The above discussion implies, however, that the spreading centres dissipate as much energy as the faults and in many cases much more. This is not very surprising for slowly spreading centres. The rifts at their centres are commonly assumed to be an indication that emplacement of new material at depth is accomplished only with difficulty and thus at the expense of considerable dissipation of energy. Fast spreading centres, however, have a profile which closely approaches that expected from buoyancy in a relaxed state. We would expect the energy dissipation to be less in this case. This presents us with a basic dilemma, since fast ridges are nearly perpendicular, implying a very small value for  $S/r_0$ . If, as we concluded, the energy dissipated by fast spreading centres is small, it follows that the energy dissipation on fast transform faults is very small, and in particular, it is much less than that dissipated on slow transform faults.

This ramification of the minimum energy argument is difficult to accept. It is possible to think of arguments to suggest that slow transform faults might dissipate more energy than fast ones or vice versa. In either case, however, one might expect that the total energy dissipation should be a function of fault length, longer faults dissipating more energy. In fact, the minimum energy argument as interpreted above would suggest that the energy loss on the Siqueiros transform fault (perpendicular configuration, fast rate) must be much less than the loss on transform fault A in the Famous area (oblique, slow rate), even though the former is more than seven times as long as the latter. The conflict would be even greater comparing transform fault A with the fossil offset of the Mendocino transform fault, for example. This leads us to suspect the validity of the minimum energy principle, at least as it is presently formulated.

We conclude, therefore, that all well-studied, segmented, slow spreading centres are oblique to their transform faults, while fast spreading centres seem to be very nearly perpendicular, and intermediate-rate centres are sometimes oblique and sometimes perpendicular. In all cases of significantly nonperpendicular ridges, the obliqueness is in the sense such that the transform faults are shorter than they would be if the ridges were perpendicular. Furthermore, the configurations at given spreading centres seem to be stable for millions of years and through changes in direction of spreading. We believe that these obser-

uations may supply a strong constraint for mechanical models of seafloor spreading and that they raise serious doubts concerning presently accepted models.

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- Vogt, P. R., Avery, O. E., Anderson, C. N., Bracey, D. R. & Schneider, E. D. *Tectonophysics* **8**, 285 (1969).
- Reid, I. & Macdonald, K. C. *Nature* **246**, 88 (1973).
- Detrick, R., Mudie, J. D., Luyendyk, B. P. & Macdonald, K. C. *Nature* **246**, 59 (1973).
- Choukroune, P., Francheteau, J. & Le Pichon, X. (in preparation).
- Luyendyk, B. P. & Macdonald, K. C. *Geol. Soc. Am. Bull.* **88**, 507 (1977).
- Macdonald, K. C. & Luyendyk, B. P. *Geol. Soc. Am. Bull.* **88**, 621 (1977).
- Ballard, R. D. & van Andel, T. H. *Geol. Soc. Am. Bull.* **88**, 507 (1977).
- Phillips, J. D. & Fleming, H. S. *Geol. Soc. Am. Bull.* (in the press).
- Macdonald, K. C. *Geol. Soc. Am. Bull.* **88**, 541 (1977).
- Bird, P. & Phillips, J. D. *J. geophys. Res.* **80**, 4021 (1976).
- Fox, P. J., Lowrie, A., Jr. & Heezen, B. C. *Deep-Sea Res.* **16** (1969).
- Fox, P. J., Schroeder, F. W., Moody, R. M., Pitman, W. C. & Hoose, P. J. *Deep-Sea Res.* (in the press).
- Sykes, L. R. *J. geophys. Res.* **72**, 2131 (1967).
- van Andel, T. H. *Mar. geophys. Res.* **1**, 274 (1971).
- Eitrem, S. & Ewing, J. *Geology* **3**, 555 (1975).
- Collette, B. J. & Rullen, R. W. *Nature* **237**, 131 (1972).
- Litvin, V. M., Marova, N. A., Rudenko, M. V. & Udinstev, G. B. *Oceanology* **12**, 631 (1972) (in Russian).
- Searle, R. C. & Laughton, A. S. *J. geophys. Res.* (in the press).
- Lonsdale, P. & Shor, A. (in preparation).
- Laughton, A. S., Whitmarsh, R. B. & Jones, M. T. *Phil. Trans. R. Soc. Lond. A* **267**, 227 (1970).
- Laughton, A. S. *Phil. Trans. R. Soc. Lond. A* **259**, 150 (1966).
- Raff, A. D. & Mason, R. G. *Geol. Soc. Am. Bull.* **72**, 1267 (1961).
- Gulf of California, McClain, J., Snysman, W. E., Lister, C. R. B., Holmes, M. L. & Heitman, C. *Geol. Soc. Am. Bull.* (in preparation).
- Macdonald, K. C. & Speiss, F. N. Deep-tow data, F. Drake Cruise (1977).
- Kligord, K. D. & Mudie, J. D. *Geophys. J. R. astr. Soc.* **38**, 563 (1974).
- Rea, D. K. *J. geophys. Res.* **81**, 1495 (1976).
- Elvers, D., Potter, K., Seidel, D. & Morley, J. NOS Sea Map BGM-1-71, N.O.A.A. (1972).
- Menard, H. W. in *Marine Geology of the Pacific* 271. (McGraw-Hill, New York, 1964).
- Mason, R. G. & Raff, A. D. *Geol. Soc. Am. Bull.* **72**, 1259 (1961).
- Molnar, P., Atwater, T., Mamerickx, J. & Smith, S. M. *Geophys. J. R. astr. Soc.* (1976).
- Johnson, G. & Vogt, P. *Geol. Soc. Am. Bull.* **84**, 3443 (1973).
- Lachenbruch, A. H. & Thompson, G. A. *Earth planet. Sci. Lett.* **15**, 116 (1972).
- Lachenbruch, A. H. *J. geophys. Res.* **81**, 1883 (1976).

## Seafloor spreading south of the Agulhas Fracture zone

FRANCHETEAU and Le Pichon<sup>1</sup> have suggested that before the break-up of Africa and South America the continental<sup>2</sup> Falkland Plateau fitted against the southern and south-eastern margin of South Africa from the southern tip of the Agulhas Bank to a position between East London and Durban. During break-up the Plateau acted as part of the South American plate and moved past Africa along a line of shear which was coincident with an initial marginal offset of approximately 610 miles. If the hypothesis is correct and normal spreading took place to the present, the original offset should be preserved in the present-day mid-Atlantic Ridge. In addition, a fracture zone should bound the southern margin of Africa and extend westwards to the mid-Atlantic Ridge, and the Cainozoic<sup>3</sup> and Mesozoic<sup>4</sup> magnetic lineaments that have been traced in the Cape Basin should be present in the Natal Valley, Transkei Basin and northern Agulhas Basin south of the fracture zone, trending perpendicular to it. New magnetic and bathymetric data presented here lend qualified support to the hypothesis and give an indication of the possible seafloor spreading history of the area.

Bathymetric, gravity and magnetic data<sup>5-7</sup> have been used to prove the existence and accurately trace the position of the Agulhas Fracture Zone<sup>5</sup> south of the southern continental margin of South Africa. West of the southern tip of the Agulhas Bank its existence has been proven<sup>5-9</sup> but it has hitherto not been accurately traced or linked with a

specific morphological feature on the sea floor. Data from numerous traverses that were run by the RV Thomas B. Davie of the University of Cape Town were used to update the bathymetric map of the South Atlantic of Simpson<sup>10</sup> in the area of the Cape Rise. The new map is presented in Fig. 1. It features a ridge that starts at 23.5°E and extends south-westwards. South of the Agulhas Bank the ridge height is variable along its length; less than 500 m above the surrounding sea floor in places and up to 3,000 m high in others, for example Mallory Seamount. West of the tip of the Agulhas Bank it becomes a low relief feature (<500 m) extending to the Richardson Seamount where it again changes character to become an elevated (>2,000 m) structure which can be followed to 5°E. The total feature is here named the Agulhas Ridge. The 68° small circle about Le Pichon's<sup>11</sup> early pole of opening for the South Atlantic has been superimposed on the map and is seen to closely coincide with the trend of the Ridge between 23.5°E and 5°E. This leads to the conclusion that the Ridge accurately reflects the westward extension of the Agulhas Fracture Zone and is the exact counterpart to the linear basement structure that marks the Falkland Fracture Zone between 28°W and 40°W (ref. 9).

There is little suitably orientated magnetic data from south of the Agulhas Fracture Zone. For the purpose of this letter a single composite track, run south of and parallel to the fracture zone from the latitude of Durban to the mid-Atlantic Ridge, was built up from two unpublished traverses of the Geological Survey of South Africa (Pr. 2a and 2b), a short section of traverse from Emery *et al.*<sup>7</sup> (Pr. 2c) and from traverse 1103 of Ladd *et al.*<sup>12</sup> (Pr. 2d). The insert in Fig. 2 shows the position of their tracks. In Fig. 2 the magnetic profile derived from measurements taken on these traverses (Pr. 2) is compared with a typical profile obtained north of the fracture zone across the Cape Basin between Cape Town and Tristan da Cunha (Pr. 1). The latter profile has been subdivided into four zones for the purposes of this discussion. Zone A includes the anomalies of the Cainozoic sequence terminating in Anomaly 34 (82–85 Myr BP, ref. 13). Following on Anomaly 34 there is a very quiet field defined as Zone B. Zone C includes an initial group of large amplitude anomalies followed by anomalies of shorter wavelength and lesser amplitude. These anomalies have been correlated with the Cretaceous Normal Polarity Epoch by some<sup>4,8,12</sup> and with the later anomalies of the Mesozoic sequence by others<sup>7,14</sup>. Zone D includes a set of high amplitude anomalies which Larson and Ladd<sup>4</sup> named the Cape Basin Lineaments and correlated with anomalies M0 to M12 (~108 to ~128 Myr BP) of the world-wide Mesozoic sequence<sup>15</sup>.

In comparing Pr. 2 with Pr. 1 it is seen that a large negative anomaly occurs on Pr. 2 in exactly in the same relative position that Anomaly 34 occurs in Pr. 1 and that though individual intercorrelations are not evident in the 'older than Anomaly 34' crust, a grouping of anomalies may be recognised that is not dissimilar to the grouping in the Cape Basin. Pr. 3 to 6 (Fig. 2) are from four closely spaced sub-parallel traverses which were run in the Natal Valley between the latitudes of East London and Durban. The clear intercorrelation that exists between individual anomalies on Profiles 2a, 3, 4 and 5 confirms that these lineaments that may be equivalent with the anomalies in Zone D in Pr. 1 in fact, trend perpendicular to the supposed local transform direction. In summary, the evidence indicates that at 85 Myr BP the original offset in the mid-ocean ridge system of 610 miles was still in existence.

On the 'young side' of Pr. 2 Ladd *et al.*<sup>12</sup> have shown that the present-day mid-Atlantic Ridge is offset by a mere 150 miles in the same sense as the original offset and that the normal Cainozoic sequence is present to at least Anomaly 24 (60 Myr BP) east of the mid-Atlantic Ridge. Beyond 24



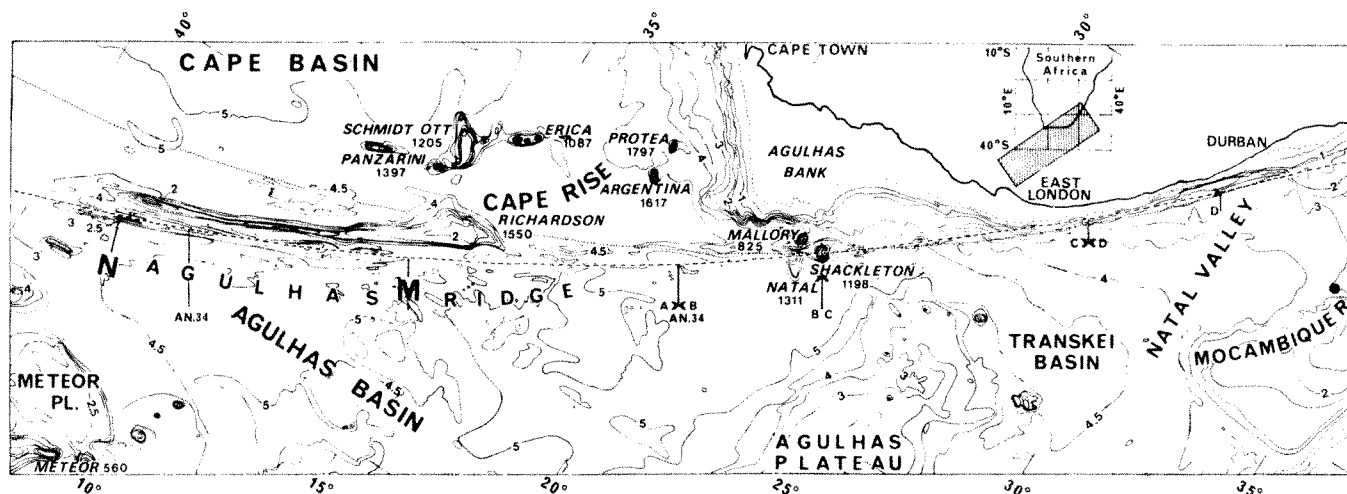


Fig. 1 A bathymetry map with a 500-m contour line interval featuring the Agulhas Ridge which marks the extension of the Agulhas Fracture Zone west of South Africa. Insert shows the area covered by the map relative to Southern Africa. The  $68^\circ$  small circle about Le Pichon's<sup>11</sup> early pole of opening at  $21.5^\circ\text{N}$ ,  $14^\circ\text{W}$  (stippled line) and the  $48.7^\circ$  small circle about  $6^\circ\text{N}$ ,  $4^\circ\text{W}$  (dotted line) are shown. The first small circle closely approximates the trend of the Agulhas Ridge west of  $23.5^\circ\text{E}$ . The second smaller circle is better approximation of the trend of the continental margin east of  $23.5^\circ\text{E}$ . The following are also shown: the boundaries between magnetic anomalies groupings A, B, C and D occurring south of the fracture zone which are correlated with a similar grouping of anomalies in the Cape Basin; the positions at which anomaly 34 is traced south of the fracture zone; positions M and N which indicate respectively the positions from which, and to which, the mid-ocean ridge is believed to have jumped at approximately 67 Myr BP.

the traverse crosses the Agulhas Fracture Zone into the Cape Basin. In the absence of any evidence of asymmetrical spreading the simplest mechanism which can be invoked to explain the difference between the present day offset of the mid-Atlantic Ridge and its original offset is a ridge jump of  $(610-150) = 460$  miles to the west sometime between 60 Myr (Anomaly 24) and 85 Myr (Anomaly 34) ago. In the central portion of Pr. 2 the data are consistent with the proposed jump having occurred at approximately 67 Myr BP (Anomaly 26): there is an apparent mirroring of magnetic lineaments about the point marked M where the ocean floor is rugged and slightly elevated above the normal level in the Agulhas Basin (Figs 1 and 2). Anomaly 34 occurs both to the east and west of M and so too, do Anomalies 31 and 32 (with less assurance). The position to which the ridge could have jumped is indicated by the point N on Figs 1 and 2.

A number of observations follow on the acceptance of this hypothesis. (1) The Agulhas Plateau is underlain by oceanic crust but is not an extinct spreading centre as suggested by Scrutton<sup>16</sup>. Assuming seafloor spreading to have started in the area at 128 Myr BP (ref. 4) the age of the crust which was involved in the tectonism and/or vulcanism

that created the Plateau centres on approximately 95 Myr BP. (2) In the block bounded by latitudes  $45^\circ$  and  $50^\circ\text{S}$  and longitudes  $0^\circ$  and  $5^\circ\text{E}$  there is an elevated plateau ( $\sim 2,500\text{m}$ ) west of M which overlies crust of similar age to the Agulhas Plateau. It may be the product of the same tectonic event. The plateau is here named the Meteor Plateau (Fig. 1). (3) The trend of the Agulhas Fracture Zone does not exactly correspond with a small circle about Le Pichon's<sup>11</sup> early pole of opening east of  $23.5^\circ\text{E}$ . The curvature of the small circle is less than the curvature of the fracture zone which has been defined as occurring at the base of the steep continental slope in the area<sup>6</sup>. A better fit is obtained by plotting the  $48.7^\circ$  small circle about a pole of opening at  $6^\circ\text{N}$ ,  $4^\circ\text{W}$ . This lends support to the suggestion of a number of workers<sup>8,14,17</sup> that during the earliest phase of opening of the South Atlantic spreading was about a pole other than the Le Pichon's early pole. The Agulhas Plateau may reflect the tectonic response of the crust to a readjustment in the spreading direction on the mid-ocean ridge when it reached  $23.5^\circ\text{E}$  in the area, 95 Myr ago. (4) M is situated near to the point where the Agulhas Ridge undergoes the dramatic change from a low narrow ridge to a complex elevated structure and N near to the

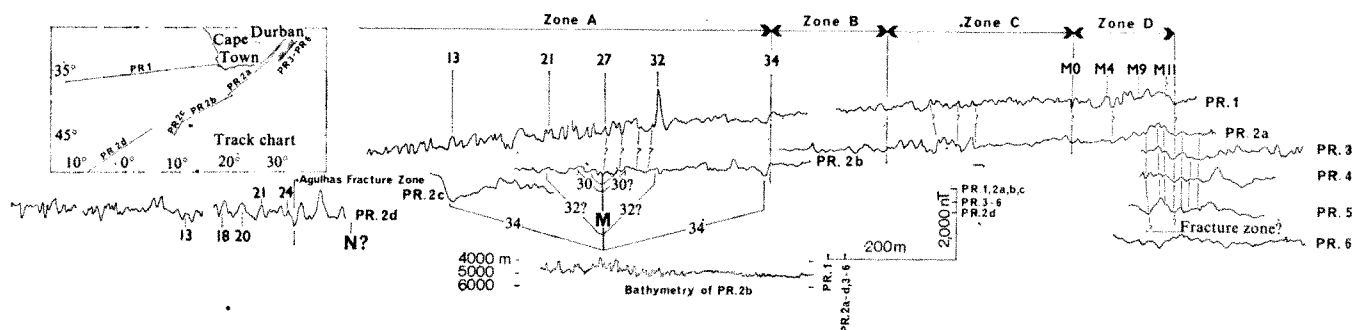


Fig. 2 Total magnetic field profiles plotted perpendicular to transform direction relative to an arbitrary datum and aligned on to the first 'apparent' seafloor spreading anomaly adjacent to the continental margin. Map insert gives positions of tracks. For data sources see text. Pr.1 is a typical profile across the Cape Basin with Mesozoic anomaly identifications following Larson and Ladd<sup>4</sup>. Pr.2 is a composite profile from south of the AFZ (Agulhas Fracture Zone). Four groupings of anomalies (Zones A, B, C and D) are shown to occur similarly on Pr.1 and Pr.2. A few tentative individual correlations are shown. Clear intercorrelations between the anomalies on Profiles 2a, 3, 4 and 5 show that the anomalies of Zone D in the Natal Valley trend perpendicular to the AFZ. An apparent symmetrical arrangement of the Cainozoic anomalies 27-34 occurs about point M south of the AFZ. A ridge jump of 460 miles from M to N is postulated (see text). The bathymetry on Pr.2b is shown.

western termination of the Ridge. This apparently suggests that during the period that vulcanism dwindled on the mid-ocean ridge at M, and was increasing at the new position of the ridge at N, it was intense along the Agulhas Fracture Zone mainly between the two points and created the elevated portion of the Agulhas Ridge.

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1. Francheteau, J. & Le Pichon, X. *Bull. Am. Ass. Petrol. Geol.* **56**, 991–1007 (1972).
2. Barker, P. F. *et al. Geotimes* **19**, 16–18 (1974).
3. Dickson, G. O., Pitman III, W. C. & Heirtzler, J. R. *J. geophys. Res.* **73**, 2087–3100 (1968).
4. Larson, R. L. & Ladd, J. W. *Nature* **246**, 209–212 (1973).
5. Talwani, M. & Eldholm, O. *Nature* **241**, 325–330 (1973).
6. du Plessis, A. & Simpson, E. S. W. *Mar. geophys. Res.* **2**, 99–110 (1974).
7. Emery, K. O., Uchupi, E., Bowin, C. O., Phillips, J. & Simpson, E. S. W. *Bull. Am. Ass. Petrol. Geol.* **59**, 2–59 (1974).
8. Rabinowitz, P. D. *Bull. Geol. Soc. Amer.* **87**, 1643–1653 (1976).
9. Rabinowitz, P. D., Conde, S. C. & La Brecque, J. L. in *Continental Margins of Atlantic Type* (ed. de Almeida) *Anais Da Academia Brasileira de Ciencias* **48**, suppl., 241–251 (1976).
10. Simpson, E. S. W. (unpublished map of National Research Institute of Oceanology, Stellenbosch, 1974).
11. Le Pichon, X. *J. geophys. Res.* **73**, 3661–3697 (1968).
12. Ladd, J. W., Dickson, G. O. & Pitman III, W. C. in *The South Atlantic* (eds Nairn, A. E. M. & Stehli, F. G.) (Plenum, New York, 1973).
13. Larson, R. L. & Pitman III, W. C. *Bull. Geol. Soc. Amer.* **83**, 364–366Z (1972).
14. Du Plessis, A. *Bull. Geol. Surv. S. Africa* (in the press).
15. Larson, R. L. & Hilde, T. W. C. *J. geophys. Res.* **80**, 2586–2594 (1975).
16. Scrutton, R. A. *Earth Planet. Sci. Lett.* **19**, 250–256 (1973).
17. Mascle, J. & Sibuet, J.-C. *Nature* **252**, 464–465 (1974).

## Ansamitocin, a group of novel maytansinoid antibiotics with antitumour properties from *Nocardia*

WE have isolated a new group of ansamycin antibiotics with potent antitumour activity, from a fermentation broth of *Nocardia* sp. No. C-15003 (N-1) and have named it ansamitocin. Structures of ansamitocin were found to be similar to maytansinoids and related maytansinoids obtained from plant sources by Kupchan *et al.*<sup>1–4</sup> and Wani *et al.*<sup>5</sup>. These compounds have strong antitumour activities, but development of production would be difficult, because plants containing maytansinoids are only harvested in tropical areas and their content in the plants is extremely low. Some attempts have been made to find a maytansinoid-producing microorganism<sup>6</sup>, but no success has been reported. We report here the microbial production, isolation and structural elucidation of these antibiotics and their antitumour activities against several experimental tumours in mice.

Strain No. C-15003 (N-1), which forms many coraemia-like bodies and motile heteromorphous cells was concluded to be a new species of the genus *Nocardia*. Fermentation was carried out at 28 °C for 4 d under aeration and with agitation, in a 2,000-l

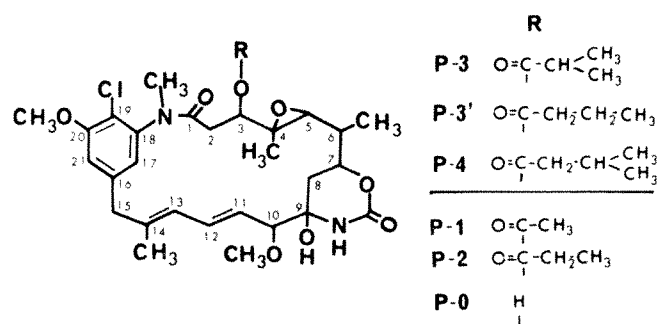


Fig. 1 Structures of ansamitocin and related compounds.

fermentor containing 1,000 l of a culture medium consisting of 5% dextrin, 3% corn steep liquor, 0.1% peptone and 0.5%  $\text{CaCO}_3$ . In the culture conditions, most of the active substances were produced in the extracellular medium.

The active substances were extracted with ethyl acetate from the culture filtrate. The extract was chromatographed on a silica gel column using a mixture of  $\text{CHCl}_3$ -MeOH and aqueous ethyl acetate to give five crystalline products, P-1, P-2, P-3, P-3' and P-4, of which P-3 and P-4 were the most abundant. The physicochemical properties of these antibiotics are as follows: P-1,  $\text{C}_{30}\text{H}_{39}\text{ClN}_2\text{O}_9$ , m.p. 235–236 °C,  $[\alpha]_D^{22} - 121^\circ$  ( $c=0.25$  in  $\text{CHCl}_3$ ),  $\text{MS } m/e$  545 ( $\text{M}^+ - \text{a}^*$ ), P-2,  $\text{C}_{31}\text{H}_{41}\text{ClN}_2\text{O}_9$ , m.p. 188–190 °C,  $[\alpha]_D^{22} - 127^\circ$  ( $c=0.35$  in  $\text{CHCl}_3$ ),  $\text{MS } m/e$  559 ( $\text{M}^+ - \text{a}^*$ ); P-3,  $\text{C}_{32}\text{H}_{43}\text{ClN}_2\text{O}_9$ , m.p. 190–192 °C,  $[\alpha]_D^{22} - 136^\circ$  ( $c=0.375$  in  $\text{CHCl}_3$ ), ultraviolet spectrum  $\gamma_{\text{max}}^{\text{MeOH}}$  nm ( $\epsilon$ ) 233 (30,250), 240 (sh. 28,450), 252 (27,640), 280 (5,750), 288 (5,700), PMR (100 MHz in  $\text{CDCl}_3$ )  $\delta$  p.p.m. 1.27 (d. 3H,  $-\text{CH}-\text{CH}_3$ ), 1.28 (d. 3H,  $-\text{CH}-\text{CH}_3$ ),  $\text{MS } m/e$  obs. 573.2471 (calc. 573.2493) ( $\text{M}^+ - \text{a}^*$ ); P-3',  $\text{C}_{32}\text{H}_{43}\text{ClN}_2\text{O}_9$ , m.p. 182–185 °C,  $[\alpha]_D^{22} - 134^\circ$  ( $c=0.11$  in  $\text{CHCl}_3$ ), PMR (100 MHz in  $\text{CDCl}_3$ )  $\delta$  p.p.m. 1.05 (t. 3H,  $-\text{CH}_2-\text{CH}_3$ ),  $\text{MS } m/e$  573 ( $\text{M}^+ - \text{a}^*$ ); P-4,  $\text{C}_{33}\text{H}_{45}\text{ClN}_2\text{O}_9$ , m.p. 177–180 °C,  $[\alpha]_D^{22} - 142^\circ$  ( $c=0.52$  in  $\text{CHCl}_3$ ), PMR (100 MHz in  $\text{CDCl}_3$ )  $\delta$  p.p.m. 1.03 (d. 6H,  $-\text{CH}-\text{CH}_3$ ),  $\text{MS } m/e$  obs. 587.2626 (calc. 587.2649) ( $\text{M}^+ - \text{a}^*$ ). The ultraviolet and infrared spectra of these antibiotics resemble each other, and no microbial product similar to these antibiotics has been reported previously. All of these antibiotics contain two atoms of nitrogen and one atom of chlorine per molecule, and their ultraviolet spectra resemble that of maytansin<sup>4</sup> obtained from a plant source.

Alkaline hydrolysis of P-1, P-2, P-3, P-3' and P-4 gave acetic, propionic, isobutyric, butyric and isovaleric acids, respectively. Analysis of the PMR spectrum and spin-decoupling studies of P-3 demonstrate that the skeletal structure is the same as that of maytansin<sup>1</sup>.

Reductive hydrolysis of each antibiotic with  $\text{LiAlH}_4$  at lower temperature gave maytansinol (P-O) (ref. 4), and acetylation of P-O with acetic anhydride in pyridine yielded P-1, maytansin<sup>4</sup>.

In the analysis of mass spectrometry, all of these antibiotics gave the same characteristic fragment peaks at  $m/e$  485, 470 and 450 as

Table 1 Antitumour activity of ansamitocin P-3 and P-4 against mouse leukaemia P388\*

Dose ( $\mu\text{g kg}^{-1}$ per injection)	Ansamitocin P-3			Ansamitocin P-4	
	MST (d)	ILS† (%)		MST (d)	ILS† (%)
50	13.0	18		13.8	25
25	24.5	123		21.5	95
12.5	19.5	77		19.5	77
6.3	18.5	68		19.8	80
3.2	17.5	59		16.0	45
1.6	15.8	44		15.5	41
0.8	13.5	23		14.5	32
0.4	11.5	5		11.5	5

\* (C57BL/6 × DBA/2)F<sub>1</sub> female mice weighing 18–22 g were inoculated intraperitoneally (i.p.) with  $10^6$  P388 cells and the test groups each consisting of five mice were treated i.p. with ansamitocin dissolved in methanol–0.85% physiological saline daily for 9 d, starting 24 h after tumour inoculation. The medium survival times (MST) of the test groups (T) were compared with that of the control group (C) consisting of 15 mice (MST = 11.0).

† Percentage increase in lifespan over controls ( $(T/C) - 100$ ).



those of maytansinoids<sup>2-4</sup>: 485 [M<sup>+</sup> - \*(a+b)], 470 [485-15(CH<sub>3</sub>)], and 450 [485-35(Cl)]; \*(a=H<sub>2</sub>O+HNCO,



The MS spectra of P-1 and P-2 were identical to those of maytansin and maytansinol propionate, respectively. We therefore concluded that P-3, P-3' and P-4 were novel ansamycin antibiotics. The structures of P-3, P-3' and P-4 correspond to those of unknown maytansinoids, maytansinol isobutyrate, -butyrate and -isovalerate, respectively. Figure 1 shows the chemical structures of ansamycin and their related compounds.

Ansamycin shows strong growth inhibitory activities against phytopathogenic fungi, dermatophytes and protozoa, but no activity against bacteria.

Antitumour activity of ansamycin against leukaemia P388 was assayed by the method of Geran *et al.*<sup>7</sup>. As shown in Table 1, P-3 and P-4 had strong antitumour activity against P388 at daily doses as low as 0.8–25 µg kg<sup>-1</sup> with a maximum effect at 25 µg kg<sup>-1</sup>. The wide effective dose range of ansamycin was very similar to that of maytansin<sup>8</sup>. Antitumour effects of ansamycin on other experimental tumours were also investigated. In the same daily dose range as that used in P388 test system, P-3 and P-4 were significantly active against B16 melanoma, sarcoma 180, Ehrlich carcinoma and P815 mastocytoma, but less active against leukaemia L1210. Typical figures of mitotic arrest of various ascites tumour cells of mice were observed microscopically 4–9 h after a single injection of these antibiotics. In accordance with this morphological observation of mitotic arrest, P-3 and P-4, like maytansin<sup>9-12</sup>, completely inhibited the polymerisation *in vitro* of tubulin purified from bovine brain<sup>13,14</sup> at a concentration of 4 µg ml<sup>-1</sup> (S. Ikeyama and M. Takeuchi, unpublished).

It should be emphasised that the discovery of a microorganism producing ansamycin opens the way for their production by fermentation. Also, the supply of ansamycin with its potent antitumour activity may contribute to the research on cancer chemotherapy.

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- Kupchan, S. M. *et al.* *J. Am. chem. Soc.* **94**, 1354–1356 (1972).
- Kupchan, S. M., Komoda, Y., Thomas, G. J. & Hintz, H. P. *J. chem. Soc., chem. Commun.* 1065 (1972).
- Kupchan, S. M., Komoda, Y., Branfman, A. R., Dailey, R. G. Jr & Zimmerly, V. A. *J. Am. chem. Soc.* **96**, 3706–3708 (1974).
- Kupchan, S. M. *et al.* *J. Am. chem. Soc.* **97**, 5294–5295 (1975).
- Wani, M. C., Taylor, H. L. & Wall, M. E. *J. chem. Soc., chem. Commun.* 390 (1973).
- Hanka, L. J. & Barnett, M. S. *Antimicrob. Agent. Chemother.* **6**, 651–652 (1974).
- Geran, R. L., Greenberg, N. H., Macdonald, M. M., Schumacher, A. M. & Abbott, B. J. *Cancer chemotherapy, Rep.* **3**, 1–103 (1972).
- Venditti, J. M., Wolpert-DeFilippes, M. K., in *Chemotherapy* **7**, 129–147 (Plenum, New York and London, 1976).
- Remillard, S., Rebhun, L. I., Howie, G. A. & Kupchan, S. M. *Science* **189**, 1002–1005 (1975).
- Schnaitman, T., Rebhun, L. I. & Kupchan, S. M. *J. Cell Biol.* **67**, 388a (1975).
- Wolpert-DeFilippes, M. K., Adamson, R. H., Cysyk, R. L. & Johns, D. G. *Biochem. Pharmac.* **24**, 751–754 (1975).

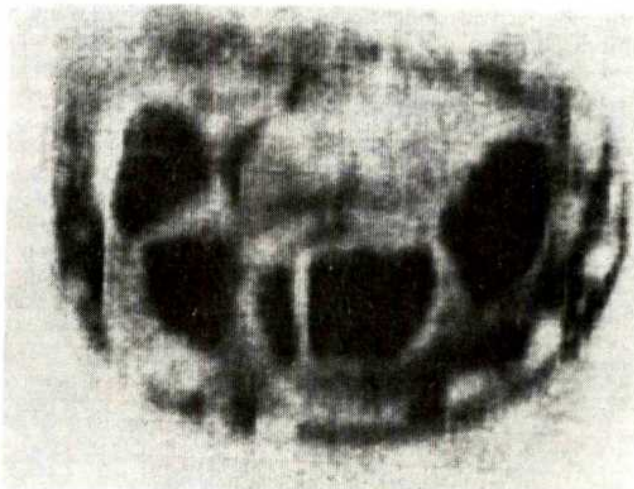
- Wolpert-DeFilippes, M. K., Bono, V. H. Jr, Dion, R. L. & Johns, D. G. *Biochem. Pharmac.* **24**, 1735–1738 (1975).
- Shelanski, M. L., Gaskin, F. & Cantor, C. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 765–768 (1973).
- Gaskin, F., Cantor, C. R. & Shelanski, M. L. *J. molec. Biol.* **89**, 737–758 (1974).

## Radiographic thin-section image of the human wrist by nuclear magnetic resonance

WE present here a detailed image showing the distribution of mobile protons in a thin section through a human wrist. The image was produced by nuclear magnetic resonance (NMR) techniques. The image consists of 128 by 128 independent picture elements and has a resolution of about 0.4 mm. For the first time images produced by NMR can be compared in quality to those produced by X-ray tomography.

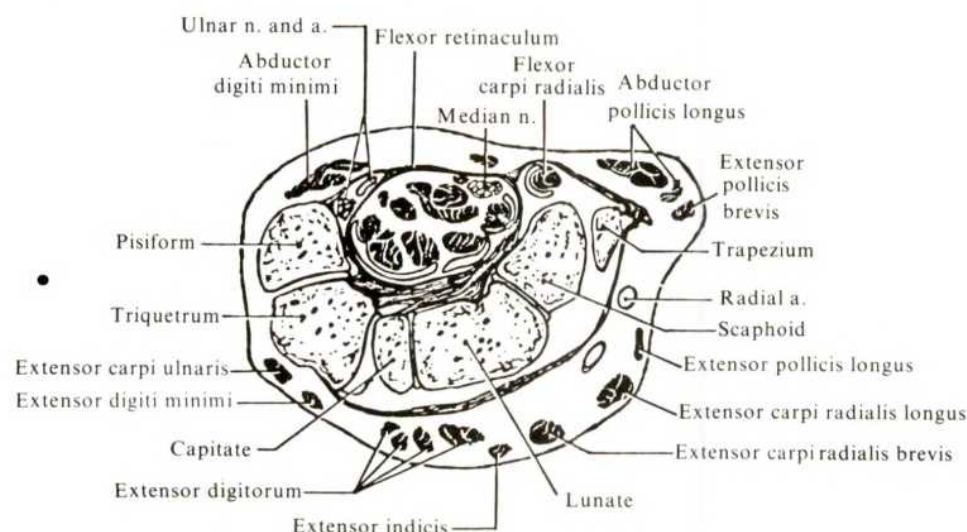
The addition of carefully controlled inhomogeneous magnetic fields makes it possible to perform NMR measurements at selected regions of heterogeneous samples and to form two-dimensional maps of the distribution of almost any property measurable by NMR<sup>1-6</sup>. The possibility of producing two-dimensional NMR images was first demonstrated by P. C. Lauterbur who used the word *zeugmatography* to denote the general method of obtaining spatial information by observing the response of a sample to a controlled inhomogeneous environment<sup>1</sup>. We have recently developed a technique for making the NMR spectrometer sensitive to a selected region of the sample<sup>3-5</sup>. By applying time-dependent magnetic fields to all of the sample except the selected region and by applying intense rf pulses and then signal averaging, we observe the signal that arises only from the selected region.

The image in Fig. 1 was produced by a combination of techniques. Two uniform orthogonal field gradients with differing time dependence were applied to the sample. The null planes of the two gradients intersect producing a line where the field is static<sup>5</sup>. This line is the selected region to which the spectrometer is sensitive. A third, static, gradient was applied along this sensitive line. The Fourier transform of the resulting NMR signal gives the distribution of the sample along the line. This distribution is displayed as beam



**Fig. 1** An NMR image of the distribution of mobile protons in a thin transverse section through the left wrist of one of the authors (P.A.B.). The image is orientated as though the author were facing you with palm upward and was taken at the level of the distal tip of the anterior horn of the lunate. The thumb was held close to the palm causing the general outline to differ slightly from the drawing in Fig. 2. Dark areas in the image indicate regions that contain high concentrations of mobile protons such as the marrow in the carpals and subcutaneous fat. Light areas indicate the presence of tissue with few mobile protons such as tendons, nerves and solid bone. Blood in the veins and arteries is light due to its motion during the imaging process.





**Fig. 2** An illustration from an anatomy textbook which shows many of the features visible in the image. This drawing represents a slightly more distal plane than the NMR image which does not show the trapezium and shows the hamate in place of the capitate. The figure is reprinted from page 141 of ref. 12 with kind permission of the authors and publishers.

intensity along a corresponding line on a storage oscilloscope. Scanning the sensitive line slowly through the sample whilst simultaneously moving the display line results in the formation of a two-dimensional image on the oscilloscope screen.

In producing Fig. 1, the spectrometer settings were such that the solid-like or rigidly-bound protons were not observed whilst those undergoing rapid thermal motion, as in 'free water', were observed. The distribution of mobile protons in Fig. 1 can be correlated with the expected structure as shown in Fig. 2. The image was recorded using a resonance magnetic field of 7 kG which gave a proton resonant frequency of 30 MHz. The two orthogonal field gradients which defined the sensitive line had a sinusoidal time dependence with frequency of  $\sim 30$  Hz and were in phase quadrature. The intensity of all three gradients was between 1 and  $10 \text{ G cm}^{-1}$ . The phase-alternated  $90^\circ$  rf pulses were  $15 \mu\text{s}$  long and were applied every 1.9 ms. The sensitive line was  $\sim 0.4 \text{ mm}$  in width and moved by  $0.4 \text{ mm}$  every 2.5 s thus scanning the object in a total of 9 min. The thickness of the slice was  $\sim 3 \text{ mm}$ .

The ability to add spatial control to NMR measurements provides a tool with considerable potential, particularly in the areas of biological and clinical research and perhaps medical diagnosis. NMR is a well established and powerful analytical technique which is capable of providing detailed information at the molecular level. In principle, localised measurements can be made of properties such as NMR relaxation times  $T_1$  and  $T_2$ , diffusion, flow, and chemical shift. We have been able to record the high resolution chemical shift spectra of the  $^1\text{H}$  and  $^{19}\text{F}$  resonances at small selected regions of the sample. The fact that NMR has no known associated hazard increases the potential of the new technique. No permanent effect has been observed from magnetic fields<sup>7</sup> and the radio frequency irradiation is several orders of magnitude less than that used for diathermy.

Spatially controlled NMR techniques should be applicable to many medical problems. Although recent measurements<sup>8-10</sup> on excised tissue have given only qualified support to the hope that NMR relaxation behaviour can be used to detect tumours, it is probable that with continued research, NMR parameters can be related to disease states. Oedema and ischaemia have been shown to affect relaxation times. One possible medical application is the imaging of fluorine in order to follow the changing chemical behaviour and physical distribution of fluorine compounds<sup>11</sup>. Although fluorine has a very low abundance in biological samples, fluorine-bearing drugs can be introduced as tracers. Further applications are also suggested by the recent use of fluoro-

carbons as blood substitutes and as breathing media in small animals.

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1. Lauterbur, P. C. *Nature* **242**, 190-191 (1973).
2. Lauterbur, P. C. *Pure appl. Chem.* **40**, 149-157 (1974).
3. Hinshaw, W. S. *Phys. Lett.* **48A**, 87-88 (1974).
4. Kumar, A., Welty, D. & Ernst, R. R. *J. mag. Res.* **18**, 69-83 (1975).
5. Hinshaw, W. S. *J. appl. Phys.* **47**, 3709-3721 (1976).
6. Mansfield, P. & Maudsley, A. A. *Br. J. Radiol.* **50**, 188-194 (1977).
7. Barnothy, M. F. (ed.) *Biologic Effects of magnetic Fields* (Plenum, New York), Vol. 1 (1964), Vol. 2 (1969).
8. Knispel, R. R., Thompson, R. T. & Pintar, M. M. *J. mag. Res.* **14**, 44-51 (1974).
9. Hazlewood, C. F., Cleveland, G. & Medina, D. J. *natn. Cancer Inst.* **52**, 1849-1853 (1974).
10. Hollis, D. P., Saryan, L. A., Eggleston, J. C. & Morris, H. P. *J. natn. Cancer Inst.* **54**, 1469-1472 (1975).
11. Holland, G. N., Bottomley, P. A. & Hinshaw, W. S. *J. mag. Res.* (in the press).
12. Gardner, E., Gray, D. T. & O'Rahilly, R. *Anatomy: A Regional Study of Human Structure* (Saunders, Philadelphia, 1975).

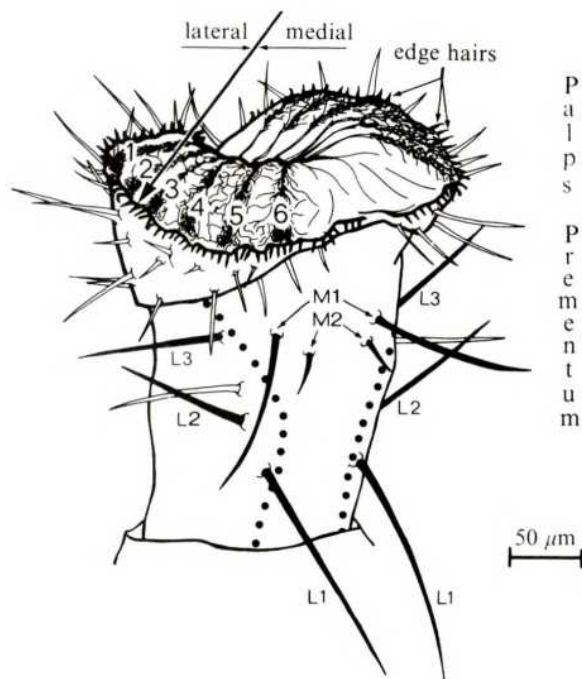
## Developmental compartments in the proboscis of *Drosophila*

A DEVELOPMENTAL compartment<sup>1</sup> in *Drosophila* is a precisely defined region of the adult fly formed by cells of a polyclone<sup>2</sup>. A polyclone consists of all the descendants of a single group of founder cells set apart at a specific stage during development. Each thoracic appendage is formed by an anterior and posterior compartment<sup>3,4</sup> which descend from nearby groups of blastoderm cells<sup>5,6</sup>. Within any part of the thorax, the boundary between these compartments does not coincide with an obvious cuticular boundary.

I have studied lineage relationships between cells forming the proboscis, a part of the adult head, by clonal and gynandromorph analysis. The results obtained indicate that each half of the proboscis (left and right) is formed from two compartments that descend from neighbouring groups of blastoderm cells. This finding suggests that the development of the proboscis may be homologous to that of the thoracic appendages, and supports the contention that compartments are a general feature of *Drosophila* development.

Proliferating cells of *Drosophila* embryos and larvae heterozygous for a dominant *Minute* mutation divide at slower rates than cells of wild-type larvae<sup>7,8</sup>. Single cells





**Fig. 1** The posterior aspect of an extended proboscis. The boundary between lateral and medial compartments is indicated by a dotted line on the prementum and by an arrow on the row of edge hairs on the labial palps. The boundary on the exterior surface of the palps is not defined since *javelin* bristles could not be reliably scored there. The six pseudotracheae have been numbered in the anterior to posterior direction. Landmark bristles (blackened in) on the prementum are as follows: the most proximal lateral bristle (L1), the next proximal (L2), the most distal lateral bristle (L3), the largest, most distal medial bristle (M1), and the medial bristle between M1 and the midline (M2). Although up to six lateral and four medial bristles may be present on each half prementum, the landmark bristles can be distinguished by their morphology and position. Nomenclature as in ref. 16. ( $\times 200$ ).

homozygous for the *Minute*<sup>+</sup> allele can be generated by X ray-induced somatic recombination. Such *Minute*<sup>+</sup> cells divide autonomously at wild-type rates<sup>7</sup> and, if made sufficiently early, form clones which fill most of the compartment in which they occur<sup>1</sup>. By examining several clones made at a specific point in development, the boundaries demarcating a compartment can be defined precisely<sup>1</sup>.

*Minute*<sup>+</sup> clones marked with *yellow*, *javelin*, and *multiple wing hairs*<sup>8</sup> were induced during the cellular blastoderm stage and detected on the proboscis by screening for *yellow javelin Minute*<sup>+</sup> bristles on the prementum (Fig. 1). All clones of more than one bristle were subsequently screened under the scanning electron microscope to determine the extent of hairs with *multiple wing hairs* phenotype. It is important to note that the proboscis is formed by the fusion

late in development of a left and a right imaginal disk. Since these imaginal disks come from distant blastoderm primordia, each half of the proboscis has been treated as a separate entity.

Table 1 summarizes the classes and frequencies of clones obtained. Of a total of 21 clones marking at least two bristles on the prementum, 12 marked only lateral bristles, nine only medial bristles, and none marked both lateral and medial bristles (Fig. 1). Scanning electron microscopy of the hairs of these marked probosces revealed a precisely defined line separating the region containing lateral bristle clones, from that containing medial bristle clones on the prementum (Figs 1, 2a). All but one clone ran along at least part of this line and 12 of 21 defined it entirely. No clones transgressed this line. In 15 cases, these clones also marked bristles and edge hairs on the labial palps (Fig. 1). The boundary separating the domains of medial and lateral prementum clones was precisely defined along the row of edge hairs. It falls between the second and third pseudotrachea (Figs 1, 2b). Six of nine lateral clones and four of six medial clones which marked the edge hairs reached this boundary and none transgressed it. The boundaries defined on the prementum and the edge hairs of the palps do not coincide with any obvious feature of the cuticle (Fig. 2a, b).

**Table 2** Sturt distances between five landmark bristles on the prementum

	M1	M2	L1	L2	L3
M1	—				
M2	0.5	—			
L1	7.8	7.2	—		
L2	7.3	6.5	1.5	—	
L3	7.5	6.6	0.5	1.5	—

Heads of 250 *y w sn<sup>3</sup> f<sup>36a</sup>/In(1) X<sup>c2</sup> w<sup>vc</sup>* (ref. 9) mosaic flies were mounted directly in Euparal (GBI Laboratories, Manchester) between two coverslips and scored for the phenotype of each of five landmark bristles on the prementum (Fig. 1), and several landmark bristles on the head. The distances between medial and lateral landmark bristles are boxed. All distances between head and proboscis landmark bristles fell within 29–35 sturts, and those between left and right proboscis landmark bristles within 23–25 sturts. The frequencies with which any of the proboscis or head landmark bristles were male fell between 55–64%.

The domains of both classes of clones account for the entire markable surface of the proboscis. In addition, the majority of clones filled or almost filled the entire domain in which they occurred. Therefore, the failure of these clones to transgress the defined boundaries indicates that each half of the proboscis is formed from two compartments. Since the clones were induced at the blastoderm stage, these compartments probably descend from two groups of founder cells set apart during or soon after this stage.

Although the two imaginal disks that form the proboscis are separate during the blastoderm stage and larval development, they may have come into contact during the intervening period of embryogenesis. If such contact occurs, *Minute*<sup>+</sup> clones should frequently mark large portions of the same compartment in both left and right proboscis halves. For example, in both anterior and posterior compartments of the first leg, the frequency of left–right overlap was greater than 30% when *Minute*<sup>+</sup> clones were induced during the blastoderm stage<sup>3</sup>. Therefore, the behaviour of each of the 21 proboscis clones with respect to the midline was examined.

All the medial clones extended to the midline of the prementum and, with one exception, did not cross it. The exceptional clone marked only one bristle and a region of hairs on the second half prementum. Similarly, of the

**Table 1** Head and proboscis clone frequencies

X-ray dose	No. of sides (left and right) screened	No. of clones having more than one marked bristle		
		Proboscis lateral	Proboscis medial	Head
500R	3,796	12	9	28
0	1,014	1	0	0

*y; Dp(1;3) sc<sup>14</sup>, y<sup>+</sup> M(3)j<sup>55</sup>/mwh jv* (ref. 9) embryos were irradiated during the cellular blastoderm stage (3.0–0.7 h after egg laying at 25 °C) with 500R of X rays (250 KV at 15 mA half-value layer 2.3 mm copper distance 3 cm, rate 530R min<sup>-1</sup>). Groups of 50–100 flies were screened blind with similar batches of unirradiated siblings. Clones on the proboscis and head were detected under the dissecting microscope ( $\times 50$ ) by the presence of *yellow javelin Minute*<sup>+</sup> bristles.



clones that marked the edge hairs, four of six medial and six of nine lateral clones extended to the midline but none crossed it. These results indicate that left and right proboscis primordia are separate until late in development. The sole case of left-right overlap may have been caused by independent clones in the left and right proboscis primordia.

To determine the proximity of the two founder groups of each half proboscis at the blastoderm stage, the distances between blastoderm cells giving rise to lateral and medial landmark bristles on the prementum were estimated by gynandromorph mapping. In this technique, the frequency (in units of 1 sturt = 1% of all gynandromorph cases<sup>10</sup>) with which two adult structures are of different sex is directly related to the distance separating the blastoderm cells from

which they are derived<sup>11</sup>. Since neighbouring adult landmarks in the same compartment can descend from the same blastoderm cell, the frequency with which they are of different sex may be extremely low. However, adjacent adult landmarks which descend from different groups of blastoderm cells cannot descend from the same cell; consequently, there must be a lower limit on the sturt distances between them. This lower limit probably represents the distance between adjacent blastoderm cells in different groups of founder cells<sup>6</sup>.

The sturt distances between each of five landmark bristles on the prementum (Fig. 1) are shown in Table 2. The distance between any lateral and any medial bristle is between six and eight sturts. The same range is observed between neighbouring adult landmarks in adjacent thoracic and abdominal compartments<sup>5,6,12,13</sup> and probably represents the distance between adjacent, but developmentally separate, blastoderm cells<sup>6</sup>. In contrast, the distance between any two lateral or medial bristles is less than two sturts, indicating that they frequently descend from the same blastoderm cell.

I conclude that each half of the proboscis is formed from two developmental compartments that descend from nearby (probably adjacent) groups of blastoderm cells. These compartments appear similar in all respects examined to the anterior and posterior compartments of the thoracic appendages. If a genuine homology exists, homeotic genes such as *engrailed* (which is required for normal development of the posterior compartments in the thorax<sup>14,15</sup>) should be similarly required in the developing proboscis.

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1. Garcia-Bellido, A., Ripoll, P. & Morata, G. *Nature new Biol.* **245**, 251-253 (1973); *Devl Biol.* **48**, 132-147 (1976).
2. Crick, F. H. C. & Lawrence, P. A. *Science* **189**, 340-347 (1975).
3. Steiner, E. *Wilhelm Roux' Archiv.* **180**, 9-30 (1976).
4. Morata, G. & Garcia-Bellido, A. *Wilhelm Roux' Archiv.* **179**, 125-143 (1976).
5. Garcia-Bellido, A. & Ferrus, A. *Wilhelm Roux' Archiv.* **178**, 337-340 (1975).
6. Lawrence, P. A. & Morata, G. *Devl Biol.* **56**, 40-51 (1977).
7. Morata, G. & Ripoll, P. *Devl Biol.* **42**, 211-221 (1975).
8. Ferrus, A. *Genetics* **79**, 589-599 (1975).
9. Lindsley, D. L. & Grell, E. H. *Carnegie Inst. & Wash. Publ.* No. 627 (1968).
10. Hotta, Y. & Benzer, S. *Nature* **240**, 527-535 (1972).
11. Garcia-Bellido, A. & Merriam, J. R. *J. exp. Zool.* **170**, 61-75 (1969).
12. Wieschaus, E. & Gehring, W. *Wilhelm Roux' Archiv.* **180**, 31-46 (1976).
13. Lawrence, P. A., Green, S. & Johnston, P. *J. Embryol. exp. Morph.* (in the press).
14. Morata, G. & Lawrence, P. A. *Nature* **255**, 614-617 (1975).
15. Lawrence, P. A. & Morata, G. *Devl Biol.* **50**, 321-337 (1976).
16. Ferris, G. F. in *Biology of Drosophila* (ed. M. Demerec.) 368-418 (Wiley, New York, 1950).

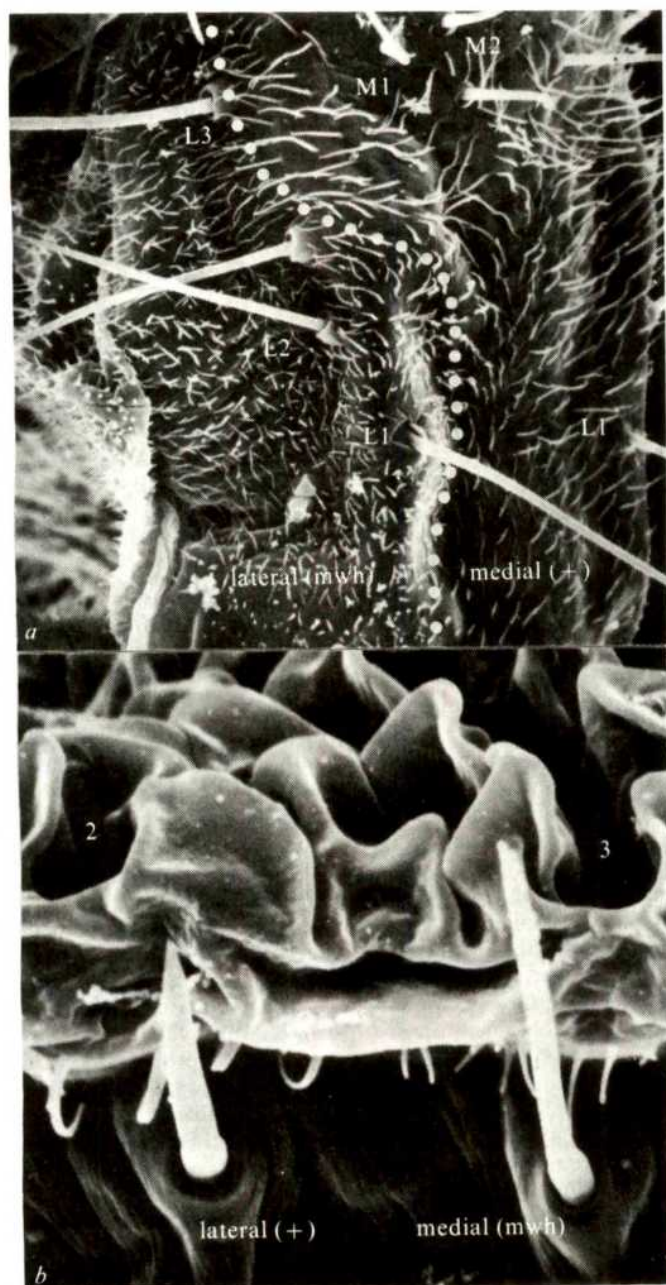


Fig. 2 Scanning electron micrographs of *Minute+ mwh* clones which define the compartment boundary *a*, on the prementum (500 $\times$ ); *b*, along the edge hairs of the labial palps (3,200 $\times$ ). The compartment boundaries, landmark bristles and pseudo-tracheae are indicated as in Fig. 1. Specimens were critical-point dried from absolute alcohol, coated with  $\sim 30$   $\mu$ m of gold, and photographed in the Cambridge S4 Stereoscan electron microscope of the Zoological Department, Cambridge.

## Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle

THE muscular dystrophies are a group of hereditary disorders manifested by a progressive wasting of the skeletal muscles. In spite of extensive studies, the nature of the primary lesion is unknown (for review see ref. 1). Because of the complex interaction between tissues, it is difficult to study this question *in vivo*. Therefore attempts have been made to investigate this question in cultures of dystrophic muscles of human or animal origin. Tissue explants as well as monolayer primary cell cultures contain, in addition to the myogenic cells, a heterogeneous cell population, the composition of which might differ in normal and dystrophic muscle cultures. It is difficult in such experiments to distinguish between properties intrinsic to the myogenic cells and effects exerted by other cell types. Indeed, previous experiments have yielded conflicting conclusions<sup>2-6</sup>. We



therefore tested the possibility of obtaining cell cultures consisting of pure populations of myogenic cells obtained from dystrophic muscles. The present report describes the isolation of a cloned population of such cells, derived from adult dystrophic mouse muscle, that can proliferate and differentiate in cell culture.

Mice of strain 129 ReJ homozygous for the recessive gene *dy* were used for the preparation of cultures. These mice manifest a syndrome very similar to human muscular dystrophy which starts early in life and progresses rapidly<sup>7</sup>. Dystrophic thigh muscles of 1–2-month-old mice were excised. After a few pieces had been removed for histological examination, the muscles were cut with scissors into pieces ~ 2 mm in size. A suspension of mononucleated cells was prepared by digestion with a mixture of 0.24% trypsin and 0.1% collagenase in  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -free phosphate-buffered saline, as described by Yasin *et al.*<sup>8</sup>. Mass cultures were plated at densities of  $5 \times 10^4$ – $5 \times 10^5$  cells per 60-mm gelatin-coated plate, and grown in Dulbecco-modified Eagle's medium supplemented with 20% foetal calf serum<sup>9</sup>. Enrichment for myoblasts was accomplished by selective serial passage of the cultures (before cell fusion), based on the observation that the attachment of suspended myoblasts to the culture plate is slower than that of most other cell types present in the cell suspension<sup>10</sup>.

C3H mice were used as donors for the serial passage of normal mouse myoblasts. Injuring the muscle by crushing with a strong forceps 2–3 d before culture preparation resulted in an over fivefold increase in the yield of mononucleated myogenic cells obtained from untreated muscle

**Fig. 1** *a*, Muscle-forming colonies in a first passage culture of cells obtained from a dystrophic muscle, plated at a density of 4,000 cells per 60-mm plate. Fixed after 13 d. ( $\times 10$ ). *b*, A section of a muscle-forming colony containing cross-striated multinucleated fibres. Fixed after 18 d in culture. ( $\times 308$ ).



**Fig. 2** A culture of  $D_1$  cells (13th serial passage), containing a network of multinucleated fibres. Fixed 13 d after plating. ( $\times 169$ ).

( $\sim 7 \times 10^6$  cells were obtained from the injured muscles of one thigh).

Mass cultures prepared from normal 2-month-old mouse muscle consisted of mononucleated cells during the first 2 d. A great proportion of them were spindle-shaped, resembling myoblasts obtained from newborn animals. Cell fusion and the formation of a network of multinucleated fibres started on the third or fourth day. In cultures prepared from dystrophic muscle, there was a great excess of fibroblastic cells over myoblasts. Most of these fibroblastic cells apparently stemmed from the connective or fat tissue which occupied a great part of the wasting muscle. Very few short multinucleated fibres were formed.

It was reported previously that outgrowths of mononucleated cells in explants of muscle obtained from dystrophic mice of the same strain failed to form multinucleated fibres: instead, the cells formed 'pseudostraps' consisting of rows of unfused cells<sup>4</sup>. Similar aggregates of cells were occasionally found in our cultures. In order to test whether the failure of cultures prepared from dystrophic muscle to differentiate was due to a lesion in the myogenic cells, or to the presence of a great excess of non-myogenic cells, cultures were plated at very low densities (500–5,000 per plate). In these conditions, colonies originating from single cells were formed and most of them were composed of non-fusing fibroblastic cells. About 5–10% of the colonies, however, consisted of homogeneous populations of spindle-shaped myoblasts. After several days these colonies contained dense networks of long multinucleated contracting fibres. The proportion of muscle-forming colonies increased markedly in secondary cultures prepared by selective cell passage (Fig. 1*a, b*).

The apparently normal morphology of the myotubes obtained in these colonies shows that at least part of the myoblasts obtained from dystrophic muscle of animals homozygous for the *dy* gene are able to proliferate, fuse and differentiate up to the stage of functional, contractile multinucleated myofibres. The poor differentiation of primary cultures prepared from dystrophic muscle thus seems to be caused by the presence of other cell types which diluted the myogenic cells or interfered in some other way with their fusion. It is, however, impossible to conclude from these experiments that in dystrophic muscle all cells belonging to the myogenic lineage are able to differentiate. The possibility that part of the non-fusing clones are abnormal muscle precursor cells cannot be excluded at present.

After establishing the existence of proliferating muscle-forming cells in the primary cultures prepared from dystrophic muscle, attempts were made to propagate these cells. Myogenic colonies obtained from primary cultures

were isolated and serially passaged. In four out of five independent experiments, proliferation decreased and the myogenic cells were lost after 3–4 passages (similar to the results obtained with normal myoblasts<sup>13</sup>). The D<sub>1</sub> line described here originated from a single myogenic colony which was isolated after the third selective passage of a primary culture plated at low density.

The cells of this line (now over 30 passages) are maintained in Dulbecco's modified Eagle's medium supplemented with 20% foetal calf serum. They proliferate as mononucleated cells with a generation time of 24 h. The main phase of cell fusion starts when confluency is reached and in the course of several days a very dense network of multinucleated fibres is formed. Fibre formation is enhanced considerably when medium supplemented with 10% horse serum is used (Fig. 2). Two to three days after the onset of cell fusion, the fibres begin to contract. Contractions are sometimes very vigorous and cause the detachment of the cell layer from the culture plate.

Two lines were established from primary cultures prepared from thigh muscle of 2-month-old normal mice. The line C<sub>1</sub> originated by selective serial passage of myoblasts obtained from thigh muscle 48 h after crush injury and C<sub>2</sub> started from cultures prepared 70 h after injury.

Formation of multinucleated fibres in chick and rat skeletal muscle cultures, is associated with a large increase in creatine kinase activity<sup>11,12</sup>. The activity of creatine kinase in the D<sub>1</sub> and C<sub>2</sub> lines was tested as a biochemical parameter for differentiation. As can be seen from Table 1, in both lines the activity of creatine kinase in differentiated cultures was over one order of magnitude higher than in cultures collected before cell fusion.

**Table 1** Creatine kinase activity in cultures of myogenic cell lines derived from dystrophic mouse (D<sub>1</sub>) and normal mouse (C<sub>2</sub>)

Cell line	Creatine kinase activity ( $\mu$ g per mg protein)	
	Mononucleated cells	Differentiated cultures
D <sub>1</sub>	80	2,160
C <sub>2</sub>	123	1,550

Cultures of mononucleated cells (48 h after plating) and differentiated cultures containing multinucleated fibres (day 5) were collected and creatine kinase was assayed as described in Shainberg *et al.*<sup>11</sup>.

These experiments show that adult dystrophic muscle of mice homozygous for the gene *dy* contain myogenic cells capable of proliferating and forming differentiated myogenic colonies. The high frequency of muscle-forming colonies in the primary cultures plated at low densities indicates that the cells capable of differentiation *in vitro* were not obtained by selection of a very rare back mutant of the *dy* gene. Rather, it shows that the progenitors of the muscle-forming colonies found in the primary cultures constitute a significant part of the mononucleated cell population of the intact dystrophic muscle. Therefore, these cells are most probably homozygous for the *dy* gene.

The ability to obtain large homogeneous cell populations which undergo differentiation in controlled culture conditions should enable comparative studies at the cellular and biochemical levels of the intrinsic properties of myogenic cells obtained from dystrophic and normal muscle. It should be stressed, however, that comparisons of the differentiation of several myogenic cell lines derived from normal rats showed some differences between lines<sup>13,14</sup>. Therefore, comparative investigations of several lines of dystrophic and normal origin will be necessary in order to distinguish between differences among individual lines acquired during their establishment and the properties related to their

genetic origin. Since the dystrophic lesion might be a disorder expressed only in fully differentiated fibres, it is also necessary for such a study to establish optimal conditions for maximal differentiation and long-term maintenance of the multinucleated fibres formed in cultures.

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- Appenzeller, O. *Arch. Neurol.* **32**, 2–4 (1975).
- Askanas, V., Shapin, S. A. & Milhorat, A. *Arch. Neurol.* **24**, 259–265 (1971).
- Bishop, A., Gallup, B., Sklate, Y. & Dubowitz, V. *J. neur. Sci.* **13**, 333 (1971).
- Parsons, R. *Nature* **251**, 621–622 (1974).
- Gallup, B. & Dubowitz, V. *Nature* **243**, 287–289 (1973).
- Powell, J. A. *Expt Cell Res.* **80**, 251–264 (1973).
- Michelson, A. M., Russell, E. S. & Harman, P. J. *Proc. natn. Acad. Sci. U.S.A.* **41**, 1079–1084 (1955).
- Yasin, R., Van Beers, G., Bulien, D. & Thompson, E. J. *Expt Cell Res.* **102**, 405–408 (1976).
- Yaffe, D. in *Tissue Culture: Methods and Applications* (eds P. F. Kruse & M. K. Patterson) 106–114 (Academic, New York, 1973).
- Yaffe, D. *Proc. natn. Acad. Sci. U.S.A.* **61**, 477–483 (1968).
- Shainberg, A., Yagil, G. & Yaffe, D. *Dev Biol.* **25**, 1–29 (1971).
- Reporter, M. C., Konigsberg, I. R. & Strehler, B. L. *Expt Cell Res.* **30**, 410–417 (1963).
- Richler, C. & Yaffe, D. *Dev Biol.* **23**, 1–22 (1970).
- Yaffe, D. & Saxel, O. *Differentiation* **7**, 159–166 (1977).

## Influence of the main histocompatibility complex on ageing in mice

CONSIDERATION of the facts of mammalian cell 'transformation' by viruses led us to the proposition that only a few genes or gene systems need necessarily be involved in the ageing process<sup>1,2</sup>. These views accord in principle with conclusions derived from more recent analyses of the genetic basis for the increase in lifespan of hominid species in the last several hundred thousand years, namely that mutations at no more than about 0.6% of the total genome could be responsible for the increase<sup>3,4</sup>. We describe here a study of ultimate lifespans (as reflected by tenth deciles of survivorship) in congenic mice which suggests that the main histocompatibility complex (MHC) is one of the gene systems involved in the control of ageing.

All vertebrate species so far investigated—birds and amphibia as well as mammals—possess an MHC: for example, the *H-2* system in the mouse and the HLA system in man. Each of these occupies a short chromosomal region of sufficient size to accommodate several hundred gene loci, although only 6–12 are as yet identified in any one species. The MHC represents a so-called 'super-gene' system, a term referring to a cluster of genes influencing the same category of functions. It influences or regulates at least the following functions<sup>5</sup>: the recognition phase of cellular immunity as manifested in the mixed lymphocyte reaction; cell-mediated lymphocytotoxicity; the development of specific suppressor cells important for immunorecognition; susceptibility to a number of viruses; susceptibility to the development of various autoimmune diseases; the ability to mount an immune response to certain specific antigens (via the immune response genes); the age-specific maturation rates, peaks and rates of decline of different immune response capacities; components of the complement system; T/B-cell collaboration (helper cells); the quantitative expression of the Thy-1 or 'theta' antigen; and levels of plasma testosterone and testosterone-binding protein. In addition gene products at the D and K loci of the *H-2* system may be required for recognition of 'self'<sup>6,7</sup>.

Ageing is associated with marked alterations in immune response capacity, particularly including *H-2* associated

Table 1 Tenth decile of survivorship and mean lifespan in male mice congenic at the H-2 chromosomal region and on three different backgrounds

Background Strain	C57BL/10 H-2 allele	1	2	3	4	5	6	7	1	2	Mean survival (weeks)	3	4	5	6	7
1 B10.AKM	m	139 ± 3.7							99 ± 3.2							
2 B10.Br/Sg	k	x	149 ± 1.1						xx	113 ± 3.8						
3 B10.PL	u	x		153 ± 1.7					xx	x	125 ± 3.1					
4 B10.A/Sg	a	x	xx		154 ± 0.4				xx	x		128 ± 4.1				
5 B10.D2/n	d	x	xx			155 ± 0.5			xx				122 ± 4.0			
6 C57BL/10	b	x	xx		x		155 ± 0.4		xx	xx	x			x	134 ± 3.0	
7 B10.RIII	r	xx	xx	xx	xx	xx	xx	170 ± 0.8	xx	xx	xx	x		xx		141 ± 3.4
<b>A</b>																
8 A.BY	b	8	9	10	11				8	9	10	11				
9 A.SW	s	114 ± 3.5							85 ± 2							
10 A.CA	f	xx	123 ± 6.6							90 ± 2.1						
11 A/Wy	a	xx	x	127 ± 3.0	134 ± 1.7				xx		85 ± 2.8	97 ± 2.5				
<b>C3H</b>																
12 C3H/HeDi	k	12	13	14					12	13	14					
13 C3H.JK	j	xx	146 ± 1.4						98 ± 3.0							
14 C3H.SW	b	xx	xx	150 ± 1.2					xx	112 ± 2.8	108 ± 3.7					

x, Indicates significant difference at the 0.05 and xx at the 0.02 probability levels.

functions, and probably also in mechanisms for distinguishing 'self' from 'non-self'. These alterations might represent primary pathogenetic processes at least in part directly responsible for ageing, or they might constitute largely secondary phenomena<sup>8</sup>. That the MHC might be fundamentally involved in ageing has been suggested on strictly immunological grounds by several workers<sup>2,9,10</sup>. Indeed, its involvement is a necessary corollary of the immunological theory of ageing<sup>8</sup>.

Direct evidence for an effect of MHC on ageing might be gained from observation of the survival patterns of congenic mice. These mice are prepared by selective breeding on any particular strain background; for example on C57BL, C3H or A strain backgrounds. Within each background, strains of mice congenic at H-2 are considered genetically 'identical' except for the relatively short chromosomal region carrying the H-2 system. In this study, therefore, we compared lifespans for both sexes of seven strains of mice congenic for H-2 on a C57BL/10 background, four on an A background, and three on a C3H background.

The 14 strains are listed in Tables 1 and 2 according to background and H-2 alleles. They were obtained either as 6-8-week-old animals, or as breeding pairs, from the Jackson Laboratory (courtesy of Dr George Snell). About 60 animals of each sex and strain were set up at two months of age, housed six per cage, and maintained on standard Purina laboratory chow and water *ad libitum*. The cages were inspected daily. The animals were allowed to live out their natural lifespans. They were not regrouped following deaths of any in the cages. The lifespan of each animal was calculated to the nearest week. Mean lifespan and standard error were determined for both sexes of each group for both the total group and for the last tenth of

survivors of a group, the so-called 'tenth decile'.

Tables 1 and 2 show the tenth decile of survivorship and the mean age of death of male and female mice for all strains. For both sexes the survival of the strains congenic upon the C57BL/10 background tended to exceed the survival of the other two background sets. This variation probably reflects the operation of some of the many non-H-2 genetic differences between the three background sets; however, we are here primarily concerned with differences within each set, as that would represent an H-2 influence. It is clear that within each set there were frequent and striking differences in strain lifespan as judged both by tenth decile survivorship and mean ages at death. For example, on the C57BL/10 background the strain B10.AKM male mouse had a significantly shorter survival than all six partner strains, and B10.AKM females shorter than five of six partner strains.

We emphasise that the mean age of death of the whole population is in fact a poor criterion of differences of physiological ageing rates between strains of mice, for it is highly susceptible to environmental influences and specific disease patterns. The ages of death of the longest-lived individual survivors in infinite populations provided in fact the best single comparative criterion of physiological ageing, as cogently argued by Sacher<sup>11</sup>, for it is in fact relatively non-susceptible to influence by environmental factors or disease processes including tumour incidence. Lacking sufficiently large or infinite populations, the mean ages at death of the tenth deciles of survivorship affords a reasonably accurate estimate for comparison and possesses similar advantages<sup>12</sup>. By this criterion as well as that of mean age at death, we noted very considerable variations among lifespans of congenic lines (Tables 1, 2). These were comparable in degree to the variations observed among

Table 2 Tenth decile of survivorship and mean lifespan in female mice congenic at the H-2 chromosomal region and on three different backgrounds

Background Strain	C57BL/10 H-2 allele	1	2	3	4	5	6	7	1	2	Mean survival (weeks)	3	4	5	6	7
1 B10.AKM	m	143 ± 2.3							101 ± 3.2							
2 B10.PL	u		145 ± 1.7							111 ± 4.0						
3 C57BL/10	b	x		148 ± 1.2					xx		120 ± 3.1					
4 B10.D2/n	d	xx	xx	xx	154 ± 0.8				x			116 ± 5.0				
5 B10.BR/Sg	k	xx	xx	xx	x	161 ± 2.1			xx	xx			125 ± 3.5			
6 B10.A/Sg	a	xx	xx	xx	xx		164 ± 2.9		xx	x				122 ± 3.9		
7 B10.RIII	r	xx	xx	xx	xx	xx	x	165 ± 1.0	xx	xx		x				129 ± 3.9
<b>A</b>																
8 A.BY	b	8	9	10	11				8	9	10	11				
9 A.SW	s	128 ± 2.3							84 ± 2.6							
10 A.CA	f		130 ± 4.3						x	89 ± 2.8						
11 A/Wy	a	xx			134 ± 3.4				xx		89 ± 3.5	94 ± 2.9				
<b>C3H</b>																
12 C3H/HeDi	k	12	13	14					12	13	14					
13 C3H.JK	j	133 ± 2.5	136 ± 1.1						94 ± 2.8	105 ± 2.6						
14 C3H.SW	b	xx	xx	151 ± 1.4					xx		109 ± 3.8					

x, Indicates significant difference at the 0.05 and xx at the 0.02 probability levels.



unrelated inbred strains of mice<sup>12</sup>. One might have expected a much greater uniformity of lifespans within each congenic set than actually observed unless the *H-2* region itself exerts a significant effect on ageing.

Since the MHC represents the master genetic control or regulatory system for immune function, particularly thymus-dependent function, which declines markedly with age, we suggest that the lifespan differences noted may reflect a complex age-related effect of the MHC on immune mechanisms. Evidence supporting this interpretation comes from separately reported studies of immune function in nine of these same strains<sup>13</sup> in which the response to different mitogens in mice from 2 to over 30 months of age were compared. On the C57BL/10 background the longest-lived strains, B10.RIII, displayed the highest response to phytohaemagglutinin throughout most of life. The shortest-lived strain, B10.AKM, displayed the lowest response. Survival patterns of the A strain mice also accorded the age-specific phytohaemagglutinin responses. Shorter lifespan could on the whole be correlated with an earlier decline in immune response capacity, as judged by these and other criteria<sup>13</sup>.

It is also possible, as suggested by Bodmer<sup>14</sup>, that a number of genes exist in the MHC region other than those determining the already recognised *H-2* antigens, and that these additional genes control the synthesis of differentiation antigens or a class of recognisers. If such be the case, an alternate or additional explanation for the observed effect of the MHC on ageing might be postulated. With regard to a possible role of the MHC in human ageing, the genetic heterogeneity of man renders the collection of useful data difficult. It has recently been noted by Yunis and Greenberg<sup>15</sup>, however, that the frequency of HLA-B8 is decreased in old cohorts of human females, suggesting the possibility of an increased mortality rate in females carrying this marker.

Our study represents one of several necessary steps in dissecting the complex genetic parameters of ageing, which are undoubtedly polygenic but are also likely to be an analysable finite number. Additional experiments, including genetic marker (MHC) identification in *F*<sub>1</sub> and back-cross studies between appropriate congenic strains, will be required to verify the apparent association between longevity and *H-2* type within any congenic set. Nevertheless, our existing data from 14 strains of mice divided into three sets congenic on three different backgrounds do strongly suggest that the MHC may be one of the principle genetic systems involved in controlling lifespan. This suggestion is in line with suggestions of Walford<sup>1,2</sup>, Cutler<sup>3</sup> and Sacher<sup>4</sup>, that ageing rates may reflect the operation of a finite number of gene systems, and not necessarily of the whole genome. The MHC may well be one of these systems.

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- Walford, R. L. *Gerontologia* **18**, 243-246 (1972).
- Walford, R. L. *Fed. Proc.* **33**, 2020-2027 (1974).
- Cutler, R. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4664-4668 (1975).
- Sacher, G. A. in *Antecedents of Man and After, Primates: Primates Functional Morphology and Evolution* (ed. Tuttle, R.) 417-441 (Mouton, The Hague, 1975).
- Walford, R. L. in *The HLA System, New Aspects* (ed. Ferrara, G. B.) 105-127 (Elsevier/North Holland, Amsterdam, 1977).
- Bevan, M. J. *Nature* **256**, 419-421 (1975).
- Doherty, P. C. & Zinkernagel, R. M. *Lancet* **i**, 1406-1408 (1975).
- Walford, R. L. *The Immunologic Theory of Aging* (Munksgaard, Copenhagen, 1969).
- Walford, R. L. *Lancet* **ii**, 1226-1229 (1970).
- Yunis, E. J., Fernandes, G. & Greenberg, W. J. in *Immunodeficiency Workshop: Birth Defects, Original Series* **11**, 85-95 (Plenum, New York, 1973).
- Sacher, G. A. in *The Lifespan of Animals, CIBA Fnd Coll. on Ageing* **5**, 115-151 (Little Brown, Boston, 1959).
- Smith, G. S., Walford, R. L. & Mickey, M. R. *J. natn. Cancer Inst.* **50**, 1195-1213 (1973).
- Meredith, P. & Walford, R. L. *Immunogenetics* (in the press).
- Bodmer, W. F. *Nature* **237**, 139-145 (1972).
- Yunis, E. J. & Greenberg, L. J. in *Genetic Effects on Aging* (ed. Harrison, D.) (National Foundation/March of Dimes, New York, in the press).

## Possibility of EB virus preferentially transforming a subpopulation of human B lymphocytes

PERMANENT lymphoblastoid cell lines can be established by 'transformation' of human lymphocytes with EB virus<sup>1,2</sup>. The lines secrete immunoglobulin (Ig) *in vitro*<sup>3,4</sup> and represent, morphologically, an intermediate stage between the resting lymphocyte and the fully developed plasma cell<sup>5,6</sup>. It has been suggested that the EB virus stimulates inactive B lymphocytes to secrete immunoglobulin and that the virus is behaving, in this respect, like a polyclonal B cell mitogen. The evidence comes from observations that most (possibly all) human B lymphocytes carry surface receptors for EB virus<sup>8</sup>, that an increase in Ig secretion is detectable within a few days of adding the virus to a culture of lymphocytes<sup>7</sup>, and that in the early stages of growth, lymphoblastoid lines established in this way secrete multiple classes of Ig heavy and light chain<sup>4,9,10</sup>. It seems, however, that only a fraction of 1% of lymphocytes in an EB virus-infected culture respond either by Ig synthesis or by proliferation and furthermore that the two events occur simultaneously<sup>7,11,12</sup>. The possibility therefore remains that EB virus does not stimulate Ig synthesis *de novo* but selectively 'transforms' (that is, induces to proliferate) that minor population of B cells which has already begun to secrete Ig. This alternative is supported by our analysis of the major classes of Ig heavy and light chains secreted by lymphoblastoid cell lines (derived from peripheral blood lymphocytes) in relation to the age and clinical status of the donors.

The data are set out in Table 1. The most striking finding is that, of fifty unselected lines derived from cord bloods, forty-nine secrete only IgM. This is at variance with the expected outcome of polyclonal stimulation of resting B cells, since the ability to synthesise cytoplasmic Ig of differing heavy chain classes in response to a polyclonal B cell mitogen seems to be acquired earlier in ontogeny than the class and subclass segregation of cell surface immunoglobulin determinants that is already well established among the circulating lymphocytes of the human foetus many weeks before birth<sup>13,14</sup>. Although IgM-secreting cells have been shown to predominate among pokeweed-stimulated human cord lymphocytes, IgG secretors form a substantial proportion in most samples and, in some cases at least, IgA-producing cells are also readily found<sup>15</sup>. It therefore seems incompatible with this mode of action of EB virus that no  $\gamma$  or  $\alpha$  heavy chains could be detected in the supernatants of any of our cord blood lines even when tested repeatedly during the early weeks following their establishment *in vitro* and even though polyclonal composition could still be demonstrated by the presence of both kappa and lambda light chains. Conversely, our findings are in keeping with the view that EB virus induces proliferation in those rare cells which are actively secreting immunoglobulin since, for the first few weeks after birth, the healthy human infant synthesises Ig exclusively of class M (ref. 16).

Adult blood lymphocytes stimulated with pokeweed mitogen typically contain populations of B cells secreting IgM, IgG and IgA respectively, in the approximate proportions of 2:2:1 (refs 15 and 17). By comparison, IgM secretors seem to be relatively over-represented among the monoclonal lines derived from healthy adults and under-represented among those from infectious mononucleosis patients (Table 1). The difference between the two groups is difficult to explain if the lines are derived from resting blood B cells, but since infectious mononucleosis is characterised

Table 1 Patterns of Ig secretion by human lymphoblastoid cell lines

Source of blood Lymphocytes*	No. of cell lines (no. of different donors)	Ig secreted†						
		G	Heavy chain		None‡	K	Light chain	
			A	M				L
Cords	Total 50(25)							
	Monoclonal Ig secretors 39(20)	0	0	49	1	30	29	1
		0	0	39	—	20	19	●
Adults (no recent infection)§	Total 79(62)							
	Monoclonal Ig secretors 66(59)	31	10	49	0	54	40	0
		20	8	38	0	39	27	0
Adults (infectious mononucleosis)	Total 19(16)							
	Monoclonal Ig secretos 17(15)	9	5	7	0	14	7	0
		7	5	5	0	12	5	0

\*All cord blood lines, all infectious mononucleosis-derived lines and all but two of the lines from other adult donors were established in our laboratory. †Ig secretion was detected by a highly sensitive and reproducible haemagglutination-inhibition technique<sup>4</sup>. In most cases supernatants had been tested on several occasions.

‡One cord-derived line (FAL<sub>1</sub>) has consistently failed to secrete any detectible Ig (ref. 4). Morphologically it is indistinguishable from other lymphoblastoid lines, and complete EB virus particles were seen in electron microscope preparations. The cells have surface Ig MK determinants (by indirect immunofluorescence) and react strongly with a rabbit antiserum specific for human B lymphoid cells.

§Although described as 'adults', three of these donors were below the age of 16 yr. The group comprised healthy individuals and those suffering from genetic (including cytogenetic) disorders or from malignant disease not affecting the lymphoreticular system.

||Lines were considered monoclonal when only a single class of Ig heavy chain and single class of light chain could be detected in the supernatant.

||The difference between these two groups in the proportion of monoclonal lines secreting M heavy chain is significant ( $\chi^2 = 4.3$ ;  $P < 0.05$ ). There are no significant differences between any group in the proportion of lines secreting K or L light chains.

by a substantial increase in serum Ig of all three major classes<sup>18</sup>, the population of Ig-secreting cells in the circulation is likely to be markedly disturbed. If this is the population which gives rise to lymphoblastoid lines, a deviation from the normal distribution of G, M and A secretors would not be unexpected. It would also provide a rational explanation for the observation that lymphoblastoid lines can be established exceptionally easily from the blood of patients in the acute or early convalescent phase of infectious mononucleosis<sup>19-21</sup> and other virus infections<sup>22,23</sup>, when the absolute number of Ig-secreting cells in the circulation is abnormally high.

Arguments based on the study of Ig secretion by monoclonal lymphoblastoid lines would, of course, be invalid if there was any suspicion of a selective advantage for the growth of cells producing Ig of a particular class, or if the commitment of a given cell and its progeny to secrete a particular class of heavy chain were unstable in the conditions of culture. On the first point, our experience has been that when multiple aliquots of 10-100 cells are taken, at an early stage of growth, from a line which is secreting comparable amounts of two immunoglobulin molecules (distinguished by heavy and/or light chain class), the subcultures ultimately give rise to approximately equal numbers of clones secreting each of the parental Ig species. Selection of the dominant clone from a newly established line therefore seems to be a random process with respect to class of Ig secreted. On the second point, in spite of one contradictory report<sup>24</sup>, it seems that lymphoblastoid cell clones secreting more than one class of heavy and/or light chain are exceptionally rare<sup>4,25</sup>, while stability of the commitment to secrete a particular class of Ig is one of the most striking features of human lymphoblastoid lines<sup>26</sup>. We have been unable to detect any change in the pattern of Ig synthesis among several hundred clones derived from lines exposed to a variety of physical and chemical mutagens, although a considerable number of other biochemical changes have been induced<sup>27,28</sup>.

The distribution of monoclonal heavy and light chains ultimately produced by lines from a particular set of donors is therefore likely to be a valid index of the relative proportions of their blood lymphocytes secreting (or committed to secrete) each class of Ig and susceptible to EB virus 'transformation'.

It is important to an understanding of the mechanism of EB virus transformation to know whether the stage of differentiation of the host B lymphocyte is a critical factor. Our conclusion, that

there is preferential, if not exclusive, transformation of those cells already secreting Ig *in vivo* (though mature plasma cells are evidently insusceptible<sup>25</sup>), seems to be more compatible with the available data than the proposition that EB virus acts as a polyclonal B cell mitogen. If the subpopulation of Ig-secreting blood lymphocytes can be isolated—for example, by velocity gradient sedimentation<sup>29</sup>—the question may be resolved.

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- Diehl, V., Henle, G., Henle, W. & Kohn, G. in *Haemic Cells In Vitro* (ed. Farnes, P.) 92 (Williams and Wilkins, Baltimore, 1969).
- Nilsson, K., Klein, G., Henle, W. & Henle, G. *Int. J. Cancer* **8**, 443-450 (1971).
- Fahey, J. L., Finegold, L., Rabson, A. S. & Manaker, R. A. *Science* **152**, 1259-1261 (1966).
- Evans, J., Steel, C. M. & Arthur, E. *Cell* **3**, 153-158 (1974).
- De Harven, E. *Cancer Res.* **27**, 2447-2464 (1967).
- Chandra, S., Moore, G. E. & Brandt, P. M. *Cancer Res.* **28**, 1982-1989 (1968).
- Rosen, A., Gergely, P., Jondal, M., Klein, G. & Britton, S. *Nature* **267**, 52-54 (1977).
- Jondal, M. & Klein, G. *J. exp. Med.* **138**, 1365-1378 (1978).
- Bechet, J. M., Nilsson, K. & Klein, G. in *Oncogenesis and Herpes Viruses* (ed. Briggs, P. M., de The, G. & Payne, L. M.) (Int. Agency Res. Cancer, Lyon, 249 1972).
- Glade, P. R. & Papageorgiou, P. S. *In Vitro* **9**, 202-215 (1973).
- Robinson, J. & Miller, G. J. *Viral* **15**, 1065-1072 (1972).
- Thorley-Lawson, D. A., Chess, L. & Strominger, J. L. *J. exp. Med.* **146**, 459-508 (1977).
- Kearney, J. F. & Lawton, A. R. *J. Immunol.* **115**, 677-681 (1975).
- Lawton, A. R., Kinkade, P. W. & Cooper, M. D. *Fedn Proc.* **34**, 33-39 (1974).
- Wu, L. Y. F., Bianco, A., Cooper, M. D. & Lawton, A. R. *Clin. Immun. Immunopath.* **5**, 208-217 (1976).
- Lawton, A. R., Self, K. S., Royal, S. A. & Cooper, M. D. *Clin. Immun. Immunopath.* **1**, 84-93 (1972).
- Janossy, G. & Greaves, M. *Transplant. Rev.* **24**, 177-236 (1975).
- Sutton, R. N. P., Reynolds, K., Almond, J. P., Marston, S. D. & Edmond, R. T. D. *Clin. exp. Immun.* **13**, 359 (1973).
- Pope, J. H. *Nature* **216**, 810-811 (1967).
- Henle, G., Henle, W. & Diehl, V. *Proc. natn. Acad. Sci. U.S.A.* **59**, 94 (1968).
- Steel, C. M. & Edmond, E., *J. natn. Cancer Inst.* **47**, 1193-1201 (1971).
- Glade, P. R., Paltrowitz, I. M. & Hirschhorn, K. *Bull. N.Y. Acad. Med.* **45**, 648-650 (1969).

23. Stevens, D. P., Barker, L. F., Pike, R., Hopps, H. E. & Meyer, H. M. *Proc. Soc. exp. Biol. Med.* **132**, 1042-1046 (1969).  
 24. Bloom, A. D., Choi, K. W. & Lamb, B. J. *Science* **172**, 382-383 (1972).  
 25. Nilsson, K. *Int. J. Cancer* **8**, 432-442 (1971).  
 26. Nilsson, K. *Haemat. Blood Transfus.* **20**, 253-264 (1977).  
 27. Poxey, S. *et al.* *Ann. Hum. Genet.* **36**, 247-266 (1973).  
 28. Arthur, M. E. *et al.* *Ann. Hum. Genet.* **39**, 33-42 (1975).  
 29. Andersson, J., Lafleur, L. & Melchers, F. *Eur. J. Immunol.* **4**, 170-180 (1974).

## Murine mesenteric and peripheral lymph nodes: a common pool of small T cells

GRISCELLI *et al.* have demonstrated<sup>1</sup> the existence of two populations of lymphoblasts with distinct migratory characteristics in the rat. One population was obtained from the mesenteric lymph nodes which migrate to the lamina propria of the small intestine and the other from peripheral lymph nodes which failed to migrate to the gut but were found in the spleen. These observations have been confirmed subsequently in the mouse<sup>2-3</sup> and in the sheep<sup>4</sup>. In mice the different migratory patterns of lymphoblasts of mesenteric or peripheral origin were shown to operate at the level of the T lymphoblast<sup>5</sup>. We have investigated whether this dichotomy extends to the migration of <sup>51</sup>Cr-labelled small T lymphocytes and have found that less than 2% of the injected small T lymphocytes localise in the small intestine (excluding Peyer's patches) and that mesenteric and peripheral small T cells migrate equally well through mesenteric, peripheral lymph nodes and Peyer's patches. There is a slight but consistent tendency for mesenteric T cells to localise more in the liver and less in the spleen than peripheral T cells. We conclude that the distinctive migration which characterises lymphoblasts in mice does not apply to small lymphocytes.

Six- to twelve-week-old mice of the inbred strains C<sub>3</sub>H/AnF (Cumberland View Farms, Tennessee) and CBA (Department of Bacteriology and Immunology, Glasgow) matched in each experiment for age and sex were used. Cell suspensions from mesenteric or peripheral (axillary, brachial and inguinal) lymph nodes were prepared by conventional methods<sup>7</sup> and T lymphocytes separated by passage through nylon wool columns as described by Julius *et al.*<sup>8</sup>. The cells in the eluate (>94% thy 1<sup>+</sup>) were labelled with (<sup>51</sup>Cr) sodium chromate<sup>7</sup> and their distribution in syngeneic recipients followed at intervals (1, 3 and 24 h) after intravenous transfer (Table 1). Little radioactivity was recovered from the small intestine, regardless of the source of the cell

inoculum, at 1, 3 (not shown) or 24 h after transfer. Mesenteric T cells (MLN-T) showed a slightly increased localisation in the Peyer's patches (at 1 h) and small intestine (at 1 and 24 h) when compared with peripheral T cells (PLN-T) but this has not been a consistently significant finding (see experiment 2 in Table 1). There was also an enhanced accumulation of MLN-T cells in the liver, which was sometimes accompanied by a decreased localisation of the MLN-T cells in the spleen and lymph nodes of the recipients. Variable percentages of damaged cells could not account for the observed differences in liver localisation, since the viability of both MLN-T and PLN-T cell suspensions before injection was greater than 97%.

In the next series of experiments we attempted to separate recirculating T cells from non-recirculating components and to enhance the differences between the migratory patterns of MLN or PLN cells by passage through syngeneic intermediary hosts. For this purpose <sup>51</sup>Cr-labelled MLN and PLN lymphocytes were injected into primary recipients which were killed 24 h later. Cells from the mesenteric or peripheral lymph nodes were then collected and the migration of the labelled cells localised in these lymph nodes was followed after intravenous transfer into secondary recipients. Since it has been shown that only a very small minority of <sup>51</sup>Cr-labelled B cells localise in the lymph nodes by 24 h (ref. 7), the population of labelled cells obtained from the lymph nodes of the intermediate recipients should consist of more than 90% T lymphocytes. The results obtained (Table 2) are in agreement with our previous experiments (Table 1). MLN cells had an increased tendency to accumulate in the liver and a decreased localisation in the spleen, compared with PLN cells. There were no differences, however, in the distribution of both MLN and PLN cells into the small intestine or Peyer's patches.

We have found that the mesenteric lymph nodes of "unprimed" mice contain consistently more pyroninophilic blast cells and incorporate more <sup>125</sup>IUDR than peripheral lymph nodes and that these cells were not removed by a nylon wool column (Table 3). Therefore, these cells could account for some of the differences observed in the migration of <sup>51</sup>Cr-labelled MLN-T and PLN-T cells. To lessen the contribution made by lymphoblasts to the migratory patterns of MLN-T and PLN-T cells these cells were depleted of lymphoblasts by velocity sedimentation through a continuous gradient of 5-20% Ficoll<sup>9</sup>. After Ficoll separation the percentage of pyroninophilic cells in both

**Table 1** Localisation of <sup>51</sup>Cr-labelled mesenteric T cells (MLN-T) and peripheral T cells (PLN-T) 1 and 24 h after i.v. transfer into syngeneic recipients

Experiment	Donor cells	Time (h)	Localisation in*						
			PLN	MLN	Spleen	Liver	Lung	PP	SI
1	PLN-T	1	3.8±0.6	8.7±1.7	31.1±3.8	8.5±0.4	6.2±0.7	2.9±0.1	1.1±0.2
	MLN-T	1	3.9±0.1	9.3±0.4	28.0±4.7	12.2±1.2†	7.6±0.2†	3.9±0.03§	1.6±0.3†
1	PLN-T	24	6.7±0.3	15.3±2.2	20.6±2.2	7.9±0.7	2.3±0.4	2.1±0.5	0.9±0.2
	MLN-T	24	6.3±0.5	17.1±1.5	22.4±2.8	12.3±1.7†	2.3±0.4	2.6±0.4	1.2±0.1†
2	PLN-T	24	4.1±0.8	10.9±0.6	18.9±0.8	5.7±0.4	1.8±0.4	1.6±0.3	0.9±0.5
	MLN-T	24	3.7±0.9	8.5±0.2§	16.7±1.0	11.2±0.2§	1.2±0.2	1.6±0.2	1.2±0.1

PP, Peyer's patches; SI, small intestine.

Nylon wool separated mesenteric and peripheral T cells were labelled *in vitro* with <sup>51</sup>Cr (sodium chromate, NEN, Chicago or Radiochemical Centre, Amersham) at a concentration of 50 µCi per 10<sup>8</sup> cells per ml for 30 min at 37 °C (ref. 7). The cells were washed three times and passed quickly through a packed column of glass wool saturated in 10% FCS containing media to remove damaged or dead cells. The viability of the cell suspensions was then assessed with the help of the Trypan blue exclusion test (0.1%) and 10<sup>7</sup> viable cells in approximately 0.2 ml injected into the lateral tail vein of syngeneic recipients. The animals were killed 1 and 24 h after cell transfer and the radioactive content of various organs was measured in a Gamma Spectrometer (Packard, USA).

\*Results are expressed as a percentage of the injected radioactive dose. Each value represents the mean (± s.d.) of 4-6 mice (C<sub>3</sub>H/AnF for experiment 1 and CBA for experiment 2). Statistical analysis was by Student's *t* test.

†*P* < 0.05.

‡*P* < 0.01.

§*P* < 0.001.



**Table 2** Secondary migration of  $^{51}\text{Cr}$ -labelled mesenteric cells (MLN) and peripheral cells (PLN) after selection in intermediate hosts

Donor cells	Time (h)	Localisation in*						
		PLN	MLN	Spleen	Liver	Lung	PP	SI
PLN	1	2.7±0.4	12.2±2.3	38.8±3.8	7.6±1.1	4.5±1.0	4.1±0.3	1.7±0.9
MLN	1	2.7±0.2	14.9±1.5†	31.2±2.2‡	9.2±0.8†	4.1±0.7	4.3±0.9	1.2±0.2
PLN	24	3.5±0.5	18.7±2.8	18.7±1.4	6.4±0.6	2.9±1.3	3.3±0.3	1.6±0.6
MLN	24	3.5±0.8	17.0±2.1	16.5±1.4†	8.1±0.9‡	2.2±0.6	3.4±0.4	2.1±0.8

Peripheral and mesenteric lymph node cell suspensions were labelled with  $^{51}\text{Cr}$  and depleted of damaged cells as described in legend to Table 1. Approximately  $2 \times 10^7$ – $3 \times 10^7$  viable cells were injected intravenously into syngeneic recipients (primary hosts). The animals were killed 24 h later and cells were collected from either peripheral or mesenteric lymph nodes. Cell suspensions were rapidly passed through glass wool columns to remove dead or damaged cells, and after this process cell viability was greater than 98% for both cell suspensions.  $10^7$  cells were injected into the tail vein of syngeneic recipients (secondary recipients). These animals were killed 1 and 24 h after cell transfer and the radioactive content of various organs was measured in a Gamma Spectrometer.

\*Results are expressed as a percentage of the injected radioactive dose ( $\sim 1 \times 10^4$ – $3 \times 10^4$  c.p.m.). Each value represents the mean ( $\pm$  s.d.) of 3–6  $\text{C}_3\text{H}/\text{AnF}$  mice. For statistical analysis see legend to Table 1.

MLN-T and PLN-T suspensions was less than 1%. Removal of lymphoblasts abolished almost completely any differences in the distribution of the two cell types (Table 4). There was still—but only 24 h after transfer—an increased accumulation in the liver and a decreased localisation in the spleen of the MLN-T cells. Note that the viability of both cell suspensions studied was superior to 97%.

than a species difference. It may be that as  $^3\text{H}$ -uridine-labelled mesenteric cells<sup>2</sup> and  $^{51}\text{Cr}$ -labelled TDL (ref. 11) migrate in high numbers to Peyer's patches in the mouse, these structures may account for much of the gut-localised  $^{51}\text{Cr}$ -labelled cells which were found in the sheep, since Peyer's patches were not removed<sup>10</sup>. It must be also noted that in the sheep studies<sup>10</sup> no attempt was made to separate large and small lymphocytes. In our investigation this

**Table 3** Size distribution and  $^{125}\text{I}$ -UdR uptake of cells in MLN and PLN cell suspensions

	Percentage cell type*			$^{125}\text{I}$ -UdR uptake†
	Pyroninophilic lymphoblast	Medium	Small	
Unseparated PLN	2.1	5.8	92.0	2,432±75
Separated PLN	1.9	6.9	92.0	2,557±81
Unseparated MLN	4.7	9.2	86.0	4,019±86
Separated MLN	5.5	8.1	86.0	4,259±64

\*Cytocentrifuge preparations of unseparated or nylon wool column separated MLN and PLN were prepared and stained with methyl green pyronin. Differential counts were performed on 500 cells under  $\times 100$  magnification.

†Nylon-wool separated MLN-T or PLN-T cells were incubated *in vitro* with iododeoxyuridine- $^{125}\text{I}$  (N.E.N., Chicago) at a concentration of 0.5  $\mu\text{Ci}$  per  $10^7$  cells per ml for 60 min at 37 °C. The cells were washed extensively and the amount of radioactivity incorporated measured in a Gamma Spectrometer. Results are expressed as c.p.m. per  $10^7$  cells. Each value represents the mean  $\pm$  s.d. of six replicates ( $\text{C}_3\text{H}/\text{AnF}$  mice).

We cannot therefore find a subpopulation of small mesenteric T cells which selectively migrates to the small intestine as happens with mesenteric lymphoblasts. Our results contrast with findings obtained in the sheep<sup>10</sup>, where large numbers of  $^{51}\text{Cr}$ -labelled T cells obtained from the intestinal lymphatics were found to migrate to the small intestine, but that T cells from the lymph draining the peripheral lymph nodes did not. There could be several reasons for these differences between mice and sheep, other

separation partially abolished the small migratory differences observed between MLN-T and PLN-T cells. Since all studies using rodents, however, have used lymph node cell suspensions, whereas those in the sheep involve the use of cells drained from the lymphatics, it is conceivable that there exists in rodents a population of T cells which recirculates exclusively through the small intestine directly into the intestinal and thoracic duct lymph, without passing through the mesenteric lymph nodes. If this is the case,

**Table 4** Localisation of lymphoblast-depleted  $^{51}\text{Cr}$ -labelled mesenteric (MLN-T) and peripheral (PLN-T) T lymphocytes 1 and 24 h after transfer into syngeneic recipients

Donor cells	Time (h)	Localisation in*						
		PLN	MLN	Spleen	Liver	Lung	PP	SI
PLN-T	1	2.2±0.4	9.9±1.2	36.9±2.7	10.0±1.5	10.3±1.0	2.6±0.5	1.4±0.4
MLN-T	1	2.5±0.7	8.9±1.0	35.3±1.2	10.7±0.8	9.3±1.0	2.4±0.5	1.5±0.6
PLN-T	24	3.7±0.6	16.7±1.3	22.2±1.7	10.4±1.4	2.0±0.8	2.2±0.3	1.4±0.9
MLN-T	24	3.3±0.5	16.2±1.9	18.6±1.0‡	12.8±1.0†	1.9±0.4	2.4±0.4	1.3±0.2

Mesenteric and peripheral T cells were first obtained by nylon wool column separation. Cells were labelled with  $^{51}\text{Cr}$  as described and depleted of any damaged cells by passage through glass wool. Approximately  $5 \times 10^7$  cells were then layered on top of a 30-ml continuous gradient of 5–20% Ficoll (in PBS supplemented with 1% FCS)<sup>9</sup>. The gradients were centrifuged at 70g for 30 min at 4 °C. The bottom 12 ml were discarded and the remaining cells were washed three times in a large volume of PBS. After separation, 97–98% of the cells were viable as determined by exclusion of 0.1% Trypan blue. Approximately  $5 \times 10^6$  to  $10^7$  cells were injected intravenously into syngeneic recipients which were killed 1 and 24 h later. The radioactive content of various organs was measured in a Gamma Spectrometer.

\*Results are expressed as a percentage of the injected radioactivity. Each value represents the mean ( $\pm$  s.d.) of 4–5  $\text{C}_3\text{H}/\text{AnF}$  mice. For statistical analysis see legend to Table 1.

then this population would be detected in the intestinal lymph but not in the mesenteric lymph nodes. This explanation is, however, unlikely for two reasons: Sprent<sup>11</sup> found that only 2.5% of <sup>51</sup>Cr-labelled murine thoracic duct lymphocytes migrate to the small intestine (allowing for localisation in Peyer's patches, see Table 6 in ref. 11) and anatomical studies of lymphatic drainage in the rat<sup>12</sup> have failed to reveal a lymphatic connection between the small bowel and cisterna chyli which does not pass through a mesenteric lymph node.

We conclude that in the mouse, mesenteric and peripheral small T lymphocytes form a common pool of cells which recirculate between the lymph nodes, spleen and Peyer's patches. The increased localisation of mesenteric small T lymphocytes to the liver may reflect the existence of slight differences in the composition of T-cell subsets between mesenteric and peripheral lymph nodes, as it has been found that <sup>51</sup>Cr-labelled Ly 23 cells have an enhanced migration to the liver when compared with Ly 1 cells or with total unseparated T cells (de Sousa *et al.* in preparation).

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1. Griscelli, C., Vassalli, P. & McCluskey, R. T. *J. Exp. Med.* **130**, 1427-1451 (1969).
2. Guy-Grand, D., Griscelli, C. & Vassalli, P. *Eur. J. Immunol.* **4**, 435-443 (1974).
3. Parrott, D. M. V. & Ferguson, A. *Immunology* **26**, 571-588 (1974).
4. McWilliams, M., Phillips-Quagliata, J. M. & Lamm, M. E. *J. Immunol.* **115**, 54-58 (1975).
5. Rose, M. L., Parrott, D. M. V. & Bruce, R. G. *Cell. Immunol.* **27**, 36-46 (1976).
6. Hopkins, J. & Hall, J. G. *Nature* **259**, 308-309 (1975).
7. Freitas, A. A. & de Sousa, M. *Eur. J. Immunol.* **5**, 831-838 (1975).
8. Julius, M. H., Simpson, E. & Herzenberg, L. A. *Eur. J. Immunol.* **3**, 645-649 (1973).
9. Tse, H. & Dutton, R. W. *J. Exp. Med.* **143**, 1199-1210 (1976).
10. Cahill, R. N. P., Poskitt, D. C., Frost, H. & Trnka, Z. *J. Exp. Med.* **145**, 420-428 (1977).
11. Sprent, J. *Cell. Immunol.* **21**, 278-302 (1976).
12. Tilney, N. L. *J. Anat.* **109**, 369-383 (1971).

## T cell receptor idiotypes are controlled by genes in the heavy chain linkage group and the major histocompatibility complex

THE molecular identity of the receptor for antigen on T cells is so far only partially defined in that the variable region of the immunoglobulin (Ig) heavy chain is involved in its specificity-determining portion<sup>1,2</sup>. All other parts of the receptor structure are at present only negatively defined: no variable region of an Ig light chain seems to be present, and the putative constant portions lack conventional immunoglobulin constant region determinants<sup>2,3</sup>.

In another set of experiments soluble T cell-derived 'factors' have been described that possess both antigen-binding specificity and antigenic determinants encoded in the I-region of the *H-2* complex<sup>4,5</sup>. These data in conjunction with the *Ir*-gene control of T cell function and the specificity differences between B and T cells suggest a model of a T cell receptor composed of one *Ig-1* coded and one *H-2* coded polypeptide<sup>6</sup>. If this model is correct, idiotypic

expression on T cells should be controlled by *V<sub>H</sub>* genes linked to allotype as well as by the *H-2* complex.

This question was investigated in mice using anti-idiotypic sera against idiotype determinants of receptors on T cell blasts activated by mixed lymphocyte reaction (MLR). The anti-sera were raised as described<sup>7</sup> by repeated injection of highly purified C57B1/6 (B6) HLR-activated AKR-T cell blasts into (AKRxB6) F1 hybrid mice. AKR responder cells were highly purified lymph node T cells (over 99.5% Ig-negative by indirect fluorescence). It is only in these circumstances that the activated T cell blasts are devoid of B cell immunoglobulin, an essential requirement for the generation of anti-idiotypic antibodies to T cell receptors only. The antiserum, which was directed against AKR receptors for B6, shortly F1a (AKRaB6), was absorbed with AKR-T cell blasts activated by SJL, to remove the previously described<sup>7</sup> 'contaminating' antibodies against determinants on T cell blast membranes not associated with antigen receptors. The anti-idiotypic specificity of the absorbed F1a (AKRaB6) was shown by three methods. (1) In indirect immunofluorescence, the absorbed antiserum stained only B6-activated AKR- and (AKRxB6) F1-T cell blasts; no staining was observed with SJL-activated AKR- and (AKRxB6) F1-T cell blasts. Con A-activated splenic AKR-T cell blasts did not stain above background. (2) In competition experiments we could show that soluble B6 alloantigens inhibited the uptake of F1a (AKRaB6) antibodies to trypsinised B6 MLR-activated AKR-T cell blasts after recovery from trypsin treatment, probably by blocking the access of the anti-idiotypic antibodies to the AKR receptors for B6 alloantigens. Only marginal blockade was observed with SJL and AKR control alloantigens. A detailed account of these experiments has been reported elsewhere<sup>7</sup>. (3) Idiotype specificity was also observed upon addition of the antiserum to MLR cultures resulting in specific inhibition of T cell proliferation. We therefore concluded that the absorbed F1a (AKRaB6) reacted specifically with AKR T cell receptors for B6 alloantigens<sup>7</sup>.

The expression of the idiotype determinants detected by F1a (AKRaB6) showed strain specificity, that is B6 MLR-activated AKR-T cell blasts expressed idiotypic whereas B6 MLR-activated SJL-T cell blasts did not (Table 1). The fact that AKR and SJL MLR responders differ in the *H-2* complex as well as by the *Ig-1* allotype (*H-2<sup>k</sup>*, *Ig-1<sup>d</sup>* and *H-2<sup>s</sup>*, *Ig-1<sup>b</sup>*, respectively) facilitated the study of the linkage of T cell receptor idiotypic expression to the *H-2* and/or *Ig-1* complexes.

Four groups of mice that differed with respect to *H-2* type and *Ig-1* allotype, were obtained by typing<sup>8,9</sup> the offspring of a backcross of (AKRxB6) F1 hybrid mice to SJL mice (Table 2): (1) *H-2<sup>s/k</sup>*, *Ig-1<sup>b/b</sup>*; (2) *H-2<sup>s/k</sup>*, *Ig-1<sup>b/d</sup>*; (3) *H-2<sup>s/k</sup>*, *Ig-1<sup>b/b</sup>* and (4) *H-2<sup>s/k</sup>*, *Ig-1<sup>b/d</sup>*. Highly purified lymph node T cells, pooled from four mice in each group, were separately activated in MLR by irradiated B6 lymph node stimulator cells. The resulting T cell blasts were purified and tested for the expression of idiotype determinants by indirect fluorescence with specifically absorbed F1a (AKRaB6). All fluorescent slides were read blind. As can be seen from Table 2, groups 1, 2 and 3 showed a low level of staining which we attribute to insufficient absorption of 'contaminating' antibodies. In marked contrast, group 4 was definitely and markedly positive. Only the mice of this group carried both the *H-2<sup>k</sup>* haplotype as well as the *Ig-1<sup>d</sup>* allotype of the idiotype-positive parental strain AKR. No intermediate level of staining was observed in the groups that carried either the *Ig-1<sup>d</sup>* allotype (2) or the *H-2<sup>k</sup>* haplotype (3). This strongly suggests that the expression of T cell receptor idiotypes on MLR-activated T cell blasts in mice is controlled by two genes, one in the major histocompatibility complex (MHC) and the other in the *Ig-1* complex. It was surprising that the percentage of stained blasts in the doubly heterozygous group 4 is similar to that of homozygous parental AKR mice, and the question of allelic exclusion is presently under investigation.

The above data have to be discussed in conjunction with two earlier observations. Hämmerling *et al.*<sup>10</sup> and Krawinkel *et al.*<sup>2</sup> reported that guinea pig anti-idiotypic antibodies to A5A idiotype bearing anti-A-CHO antibodies of A/J mice react with helper T cells that possess A-CHO specificity, and that this reaction was

**Table 1** Strain specificity of T cell receptor idiotype expression on B6 MLR-activated AKR and SJL responder T cell blasts.

Responder	T cell blasts from MLR*	Stimulator	Positive responder T cell blasts (%)† with F1a (AKRaB6) anti-idiotypic serum‡
AKR (H-2 <sup>k</sup> , Ig-1 <sup>d</sup> )		B6	35.1%
SJL (H-2 <sup>s</sup> , Ig-1 <sup>b</sup> )		B6	5.0%

\*Responder cells were nylon wool column passaged nonadherent lymph node cells (over 99.5% immunoglobulin negative by immunofluorescence; 38% (AKR) and 48% (SJL) recovery of cells from the nylon wool column). Stimulator cells were 3300 R irradiated lymph node cells. The conditions of the MLR and the nylon wool column separation have been described<sup>1,3</sup>. AKR (responder, R), B6 (stimulator, S); stimulation index, S.J.:616; over 99% blasts. SJL (R), B6 (S): S.J. 8.5; 91% blasts (over 99% T cells).

†Between 200 and 300 T cell blasts were counted. Blasts were recovered from the MLR by the Ficoll Urovison technique as described<sup>1,3</sup>. Blast cells were defined as cells with a diameter at least twice that of a small lymphocyte, with a smaller nuclear to cytoplasmic ratio than small lymphocytes and with a nonsegmented nucleus (in distinction to nonlymphoid cells). Ig<sup>+</sup> T<sup>+</sup> blasts were detected by treating the cells simultaneously with rabbit anti-mouse T cell serum<sup>1,4</sup> (dilution 1/400) and the mouse antiserum detected by tetramethyl-rhodamine isothiocyanate conjugated sheep anti-rabbit immunoglobulin G and fluorescein isothiocyanate conjugated sheep anti-mouse immunoglobulin G. All sera as well as all fluorescent conjugates were centrifuged at 16,000g for 10 min immediately before use. The exact procedure for fluorescent staining, preparation and labelling of anti-Ig antibodies, and fluorescence microscopy have been described<sup>7,13,15</sup>.

‡The F1a (AKRaB6) anti-idiotypic serum was raised as described<sup>7</sup>. The antiserum was rendered specific by absorption with SJL MLR-activated AKR-T cell blasts, and used at a final dilution of 1/20. Control staining with normal (AKRxB6) F1 serum was below 1.5%.

restricted to T cells of those strains whose antibodies expressed the A5A idiotype. Since A5A idiotype expression is genetically linked to *Ig-1*<sup>9</sup> and unlinked to *H-2*<sup>10</sup>, it was concluded from these studies that T cell receptor idiotype expression is controlled by genes in the *Ig-1* complex and not by genes in the MHC. Because in these studies the anti-idiotypic antibodies were produced against an antibody molecule, whereas the anti-idiotypic antibodies used in the present experiments are made against T cell receptors directly, the two sets of data are not contradictory. Antisera produced against antibody molecules are not expected to react with T cell idiotype determinants other than those shared with immunoglobulins and, consequently, cannot reveal any linkage to genes other than to those encoding immunoglobulin polypeptide chains.

Conversely, anti-idiotypic antisera produced against T cell receptors are expected to reveal idiotype determinants unique to

unaltered set with allo-reactivity. Thus, strains of mice that differ in their *H-2* antigens would possess different anti-self sets and, consequently, different allo-reactive sets. Phenotypically this situation clearly results in the linkage of genes controlling allo-reactive receptors to the *H-2* complex. However, as the number of allo-antigens by far exceeds the number of self-antigens, it is reasonable to assume that the receptor sets with allo-reactivity partially overlap between different inbred strains. Thus, certain strain combinations with different *H-2* types should share alloantigen receptors, and in these cases no *H-2* linkage would be observed. This may be an explanation for the results of Binz *et al.*<sup>11</sup> mentioned above.

A more direct way to account for MHC control of T cell idiotypes is that genes in *H-2* directly encode peptides that are part of the receptor structure, as has been discussed in detail before<sup>6</sup>. This participation can take place either by a physical

**Table 2** Linkage of T cell receptor idiotype to the *H-2* and the immunoglobulin-1 locus

Responder†	T cell blasts from MLR*	Stimulator	Positive responder T cell blasts with F1a (AKRaB6) anti-idiotypic serum (%)
Group 1 (H-2 <sup>s/s</sup> , Ig-1 <sup>b/b</sup> )		B6	6.2%
Group 2 (H-2 <sup>s/s</sup> , Ig-1 <sup>b/d</sup> )		B6	6.6%
Group 3 (H-2 <sup>s/k</sup> , Ig-1 <sup>b/b</sup> )		B6	6.3%
Group 4 (H-2 <sup>s/k</sup> , Ig-1 <sup>b/d</sup> )		B6	40.6%

The experimental conditions for immunofluorescence are the same as in Table 1. All fluorescent slides were read blind. Control staining with normal (AKRxB6) F1 serum: 1.7% (group 1), 0.6% (group 2), 0.6% (group 3), and 4.6% (group 4).

\*Responder cells were nylon wool column passaged nonadherent lymph node cells (over 99% immunoglobulin negative by immunofluorescence; 40% (group 1), 64% (group 2), 48% (group 3), and 56% (group 4) recovery of cells from the nylon wool column. Group 1 (R), B6 (S): S.J. 143; group 2 (R), B6 (S): S.J. 319; group 3 (R), B6 (S): S.J. 64; group 4 (R), B6 (S): S.J. 128.

†Each group consisted of cells from a pool of 4 mice *H-2* typed and allotyped as described<sup>8,9</sup>.

T cells, should they exist. In this context, Binz and Wigzell have published a series of experiments showing that anti-idiotypic antibodies against rat T cell alloantigen-receptors do not reveal any idiotype determinants unique to T cells and, consequently, do not reveal any linkage of T cell idiotype-controlling genes to genes other than those controlling immunoglobulin heavy chains<sup>1,11</sup>. These data suggested that the T cell receptor is constructed from *VH* regions only.

In contrast, the data presented in this paper clearly show that T cell receptor idiotypes are controlled by two unlinked genes. One of these genes is located in the heavy chain linkage group (*Ig-1* complex) and all the available evidence<sup>1,2,3,10,11</sup> suggests that this directly encodes a heavy chain *V* region peptide that is part of the T cell receptor. The control exerted by the second, *H-2* linked, gene(s) can be envisaged in at least two different ways. MHC-coded antigens could play an essential role in the generation of diversity in the sense suggested by Jerne<sup>12</sup> who proposed that the immunological repertoire is composed of two sets of specific receptors, one derived by mutation and selection from a set of receptors with anti-self specificity, and the other a relatively

association of the *H-2* product with *VH*, or by the existence of a separate class of *H-2* coded receptors in addition to the *VH*-containing receptors. The latter possibility seems unlikely because in the present experiments no intermediate phenotypes have been observed in the mice carrying either the appropriate *H-2* allele or the appropriate allotype.

For the moment, we cannot distinguish between an indirect regulatory influence of *H-2* antigens by selection of a given receptor repertoire, and a direct coding of genes in the *H-2* complex for parts of the T cell receptor. We may, however, generalise in concluding that the T cell repertoire differs from that of B cells, either by virtue of physical differences in the combining sites or by virtue of different routes of selection in ontogeny.

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1. Binz, H. & Wigzell, H. *Cold Spring Harbor Symp. quant. Biol.* **61**, 275-284 (1976).
2. Krawinkel, U., et al. *Cold Spring Harbor Symp. quant. Biol.* **61**, 285-294 (1976).
3. Eichmann, K. in *The Immune System: Genes and the Cells in which they Function* (eds Sercarz, E., Herzenberg, L. & Fox, F.) (Academic Press, New York, in the press).
4. Taussig, M. J., Munro, A. J., Campbell, R., David, C. G. & Stained, N. A. *J. exp. med.* **142**, 694-712 (1975).
5. Tada, T., Taniguchi, M. & David, C. G. *J. exp. med.* **144**, 713-731 (1976).
6. Rajewsky, K. & Eichmann, K. in *Contemporary Topics in Immunobiology - T cells* (ed. Stutman, O.) 69-112 (Plenum Press, New York, 1977).
7. Krammer, P. H. *J. exp. med.* (in the press).
8. Wettstein, P. J., Krammer, P., Nowinski, R. C., David, C. S., Frelinger, J. A. & Shreffler, D. C. *Immunogenetics* **3**, 507-516 (1976).
9. Eichmann, K. & Berek, C. *Eur. J. Immun.* **3**, 599-601 (1973).
10. Hammerling, G., Black, S. J., Berek, C., Eichmann, K. & Rajewsky, K. *J. exp. Med.* **143**, 861-879 (1976).
11. Binz, H., Wigzell, H. & Bazin, H. *Nature* **264**, 639-642 (1976).
12. Jerne, N. K. *Eur. J. Immun.* **1**, 1-9 (1972).
13. Nagy, Z., Elliott, B. L., Nabholz, M., Krammer, P. H. & Pernis, B. J. *J. exp. Med.* **143**, 648-659 (1976).
14. Sauter, D., Anckers, C. & Bron, C. *J. Immun.* **113**, 617-624 (1974).
15. Krammer, P. H., Citronbaum, R., Read, S. E., Forni, I. & Lang, R. *Cell Immun.* **21**, 97-111 (1976).

## Depression of macrophages in mice drinking hyperchlorinated water

WE have encountered a problem with the collection and *in vitro* activation of peritoneal exudate macrophages (PEM) from mice, which we can now associate with high levels of chlorine in the drinking water. Historically the chlorination of drinking water in colonies of experimental rodents was carried out in order to avoid the so-called early death syndrome in lethally irradiated animals, which is due to pathogenic enteric bacteria such as *Pseudomonas*. The recommended level of chlorine necessary to achieve this goal is 12-16 parts per million (p.p.m.)<sup>1</sup>. Because of an unusually high incidence of *Pseudomonas* within a few breeding units of our animal facility, the chlorination level of the drinking water was increased to 25-30 p.p.m. We found that this hyperchlorination had an adverse effect on an important host defense mechanism, that is the macrophage system, whose primary function is to eliminate microbial pathogens<sup>2,3</sup> and neoplasms<sup>3,4</sup>.

Macrophages that normally reside in the peritoneal cavity and those that are induced to accumulate there by the intraperitoneal (i.p.) injection of inflammatory agents are not cytotoxic to tumour cells *in vitro*<sup>5-8</sup>. Such PEM can be rendered tumoricidal (TM) by bacterial products, endotoxins, pyran copolymer and double-stranded RNA, chronic infection of animals with obligate intracellular bacteria, or certain protozoa<sup>7,8</sup>. Macrophages can also be rendered tumoricidal by incubation with soluble mediators (lymphokines) released by antigen- or mitogen-stimulated lymphocytes, referred to as 'macrophage activating factor' (MAF)<sup>7-11</sup>.

Tumoricidal activity can also result as a consequence of a host immune response against syngeneic tumours *in vivo*<sup>5,6,12</sup>. Recently, we found that PEM collected from tumour-immunised mice was not tumoricidal *in vitro*, and PEM from normal mice could not be rendered tumoricidal *in vitro* by incubation with MAF. Since the collection and culture of PEM, and cytotoxicity assays using PEM are influenced by a variety of factors<sup>4,6,7</sup>, we first attempted to overcome our difficulties by varying the media, serum lot, type of inflammatory agent, and so on; all to no avail. We then examined the daily records of our animal facility and noticed that the levels of chlorine in the drinking water had been increased from 12-15 p.p.m. to 25-30 p.p.m. This increase seemed to coincide with the emergence of our difficulties. We therefore set out to investigate the possibility that hyperchlorination of drinking water could adversely affect murine PEM.

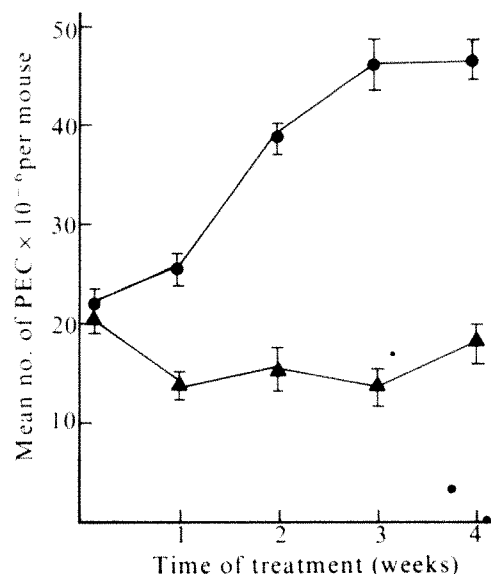
C57BL/6N female mice weaned at 3-4 weeks of age were given sterile (and free of *Pseudomonas*) tap water (0.5-1.0 p.p.m. chlorine) for 2 weeks. When the mice were 5-6 weeks old they were divided into two groups. The first group

received hyperchlorinated drinking water (25-30 p.p.m.) and the second group continued on tap water and served as controls. Chlorine and chloramine were assayed by the *O*-tolidine method<sup>13</sup> by which the total chlorine is measured ( $\text{OCI}^-$ ,  $\text{HOCl}$ ,  $\text{NH}_2\text{Cl}$ ,  $\text{NHCl}_2$  and  $\text{NCl}_3$ ). Chlorinated drinking water was prepared (as is the routine procedure in our animal colony) twice weekly by adding a solution of sodium hypochlorite to tap water. At weekly intervals groups of five mice each were injected i.p. with 2 ml of thioglycollate broth. Individual mouse PEC differential and total count was carried out 5 d later. The results of a representative experiment are shown in Fig. 1. At the start of this experiment and just before treatment, the mean number of PEC per mouse was  $21 \pm 4 \times 10^6$ . One week later the number of PEC obtained from mice receiving hyperchlorinated water had decreased to  $13 \pm 2 \times 10^6$  per mouse, whereas control mice yielded  $25 \pm 3 \times 10^6$  PEC ( $P < 0.001$ ). On consecutive weeks, the PEC yield from control mice increased, but the PEC yield from mice receiving hyperchlorinated water remained low.

At each week, PEM from mice receiving hyperchlorinated water or tap water were assayed for *in vitro* tumoricidal properties. The adherent PEM cultures were incubated *in vitro* with supernatants obtained from cultures of rat lymphocytes stimulated with Sepharose-bound concanavalin A (Pharmacia) which were previously shown to contain MAF activity (Con A-MAF)<sup>10</sup>. Macrophage-mediated cytotoxicity *in vitro* was measured by release of radioactivity from pre-labelled target cells<sup>5,10,12</sup> as described in the legend to Table 1. Tumour cell killing *in vitro* was measured by release of radioactivity ( $^{125}\text{I}$ -UdR) from pre-labelled syngeneic tumour cells, the B16 melanoma and ultraviolet-induced fibrosarcoma. Results of a representative experiment are shown in Table 1.

PEM from mice receiving tap water or hyperchlorinated water were not significantly cytotoxic to either syngeneic tumour target, which is in agreement with our previous findings<sup>5,10,12</sup>, and those of others<sup>3,4,6-9,16,20</sup>. The PEM from mice receiving tap water were cytotoxic to both syngeneic tumour targets following their treatment with Con A-MAF. The levels of specific cytotoxicity differed slightly from week to week and ranged from 43 to 68% for the B16 melanoma and 62-76% for the UV-112 fibrosarcoma ( $P < 0.001$ ). In contrast, PEM collected from hyperchlorinated mice exhibited significantly lower levels of cytotoxicity ( $P < 0.01$ ) during the first two weeks of treatment

Fig. 1 Mean no. of peritoneal exudate cells collected from mice receiving tap (0.5 p.p.m. chlorine ●) or hyperchlorinated (25-30 p.p.m. ▲) drinking water. At any time point, the differences between the groups were highly significant ( $P < 0.001$ ).



(28–31% for the B16 melanoma and 24–48% for the UV-112 fibrosarcoma). By the third week of treatment, these PEM (even though treated with Con A-MAF) were not tumoricidal.

These studies show that the addition of high levels of chlorine in the drinking water of mice produces profound alterations in numbers of PEM and tumoricidal function. The hyperchlorination of drinking water in facilities housing laboratory animals is a common occurrence. The effects of 25–30 p.p.m. of chlorine become apparent within a matter of weeks. It is possible that the lower level (10–15 p.p.m.) of chlorine also exerts such effects, but over a much longer period of time. Supporting this possibility is the report that urban water (chlorinated at 2–4 p.p.m.) filtered by reverse osmosis and used in long-term haemodialysis of human patients was shown to cause acute haemolytic anaemia. Chlorine compounds brought about denaturation of haemoglobin by direct oxidation and also by the inhibition of the direct oxidative pathway, hexose monophosphate shunt (HMPS), of red blood cell (RBC) metabolism. This damage to RBC in haemodialysed patients was found to be cumulative over several periods of dialysis<sup>14</sup>.

The mechanism by which hyperchlorinated water affects murine macrophages is unknown. Several possibilities may be considered. The vacuolar system of macrophages is probably involved in the mechanism of cytotoxicity<sup>8,15</sup>. Inhibition of macrophage lysosomal enzymes by Trypan

blue and stabilisation of lysosomal membranes by hydrocortisone have been shown to suppress macrophage cytotoxic activity<sup>16</sup>. Moreover, the translocation of lysosomes from tumoricidal macrophages into susceptible target cells has been demonstrated by phase contrast<sup>16</sup> and electron microscopic studies<sup>17</sup>. Lymphokine-treated macrophages, which have enhanced bactericidal capacity<sup>18</sup>, were found to have a 4- to 8-fold increase in glucose oxidation compared to untreated control macrophages. Nearly all the observed glucose oxidation was attributed to metabolism through the direct oxidative pathway (HMPS)<sup>19</sup>. An inhibition of HMPS metabolism in macrophages by chlorine compounds<sup>14</sup> could perhaps reduce their tumoricidal activity. Chlorine compounds may also affect macrophages indirectly. Pathological conditions that produce large numbers of damaged RBC, haemoglobin or haemoglobin degradation products within the macrophage vacuolar system (lysosomes) have now been shown to suppress macrophage tumoricidal activity<sup>15</sup>. As stated above, chlorine compounds have been shown to cause severe methaemoglobinemia and haemolysis<sup>14</sup>.

Regardless of the mechanism by which chlorinated water suppresses macrophages, the consequences of this suppression could be quite far reaching. The macrophage is thought to play a major part in host defence against neoplasia<sup>2–12</sup>. Further, substances known to depress macrophage function such as silica, carageenan and Trypan blue are reported to decrease host resistance against transplantable tumours<sup>20,21</sup>. These findings and the experiments reported here raise the possibility that host resistance against neoplasms may also be compromised by hyperchlorinated drinking water.

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1. Simmons, M. L. & Brick, O. J. in *The Laboratory Mouse Selection and Management* (ed. Hollander, A.) 67–69 (Prentice Hall, Englewood Cliffs, 1969).
2. Mackaness, G. B. in *Infectious Agents and Host Reactions* (ed. Mudd, S.) 62–78 (Saunders, Philadelphia, 1970).
3. Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *Nature new Biol.* **235**, 48–50 (1972).
4. Hibbs, J. B., Jr, Taintor, R. R., Chapman, H. A. & Weinberg, J. B. *Science* **197**, 279–282 (1977).
5. Fidler, I. J. *J. natn. Cancer Inst.* **55**, 1159–1163 (1975).
6. Russell, S. W. & McIntosh, A. T. *Nature* **268**, 69–71 (1977).
7. Evans, R. & Alexander, P. in *Immunobiology of the Macrophage* (ed. Nelson, D. S.) 536–573 (Academic, London, 1976).
8. Hibbs, J. B., Jr *J. natn. Cancer Inst.* **53**, 1487–1492 (1974).
9. Piessens, W. F., Churchill, W. J., Jr & David, J. R. *J. Immun.* **114**, 293–299 (1975).
10. Fidler, I. J., Darnell, J. H. & Budmen, M. B. *Cancer Res.* **36**, 3608–3615 (1976).
11. Fidler, I. J., Darnell, J. H. & Budmen, M. B. *J. Immun.* **117**, 666–673 (1976).
12. Kripke, M. L., Budmen, M. B. & Fidler, I. J. *Cell. Immun.* **30**, 341–352 (1977).
13. Sawyer, C. N. & McCarthy, P. L. *Chemistry for Sanitary Engineers* (McGraw, Hill, New York, 1964).
14. Eaton, J. W., Kolpin, C. F., Swofford, J. S., Kjellstrand, C. M. & Jacobs, H. S. *Science* **181**, 463–464 (1973).
15. Weinberg, J. B. & Hibbs, J. B., Jr *Nature* **269**, 245–247 (1977).
16. Hibbs, J. B., Jr *Science* **184**, 468–471 (1974).
17. Bucana, C. et al. *Cancer Res.* **36**, 4444–4458 (1976).
18. Nathan, C. F., Karnovsky, M. L. & David, J. R. *J. exp. Med.* **133**, 1356–1364 (1971).
19. David, J. R. in *Progress in Immun.* (ed. Amos, B.) 399–412 (Academic, New York and London, 1971).
20. Hibbs, J. B., Jr *Transplantation* **19**, 77–81 (1975).
21. Keller, R. J. *J. natn. Cancer Inst.* **57**, 1355–1361 (1976).

**Table 1** *In vitro* cytotoxicity mediated by MAF-treated and untreated macrophages from C57BL/6 mice receiving hyperchlorinated or tap drinking water

Week of treatment	Drinking water*	% Macrophage-mediated Cytotoxicity † against	
		B16 Melanoma	UV-112 Fibrosarcoma
1	Tap	55‡	76‡
	Hyperchlorinated	28§	48‡
2	Tap	68‡	70‡
	Hyperchlorinated	31§	24§
3	Tap	43‡	62‡
	Hyperchlorinated	0	0
4	Tap	48‡	72‡
	Hyperchlorinated	0	0

\*Tap water: 0.5–1.0 p.p.m. of chlorine. Hyperchlorinated: 25–30 p.p.m. of chlorine.

†PEC (suspended in supplemented media) were plated into 60 × 15 mm plastic dishes at a concentration of  $2 \times 10^6$  per dish. Thirty min after incubation at 37 °C, non-adherent PEC were removed by washing with media, and fresh media was added. The cultures were then incubated for an additional 24 h. At this time, all the adherent cells from mice receiving tap or hyperchlorinated water were phagocytic and morphologically resembled macrophages. The plating efficiencies and differential counts of PEC collected from the two treatment groups were similar throughout the experiments. The B16 melanoma and fibrosarcoma UV-112 tumour target cells from the C57BL/6 mouse strain were labelled *in vitro* for 24 h with 0.3 µCi/ml of <sup>125</sup>I-UdR (200 mCi/µmol<sup>-1</sup>; New England Nuclear). The tumour cultures were washed with media to remove unincorporated radioactive label and cells were collected.  $1 \times 10^4$  labelled target cells were plated alone or were added to the Con A-MAF treated or untreated macrophage cultures, making an initial ratio of 100:1 adherent PEM to target cells. After 24 h, the triplicate cultures were washed and re-fed with media to remove target cells that did not plate. On day 5, the cultures were washed twice to remove non-adherent cells, and the remaining viable cells were lysed with 1 ml 0.5 M NaOH. The lysate and two rinses were combined and counted in a gamma counter. The plating efficiencies of tumour cells plated alone or on macrophage cultures have been previously shown to be similar<sup>8,10</sup>. The % cytotoxicity in the macrophage assays was computed with the following formula: [(c.p.m. of target cells with normal macrophages – c.p.m. of target cells with TM)/c.p.m. of target cells with normal macrophages] × 100. The statistical significance of differences between groups was tested with Student's two-tailed *t*-test.

‡*P* < 0.001.

§*P* < 0.02.

## *In vitro* culture reduces immunogenicity of pancreatic endocrine islets

HUMAN pancreatic transplantation results, using the whole organ, have been disappointing and interest has been focused recently on the promising results obtained by trans-

planting isolated endocrine islets. Islet cell is transplantation can restore the diabetic animal successfully to a state of normoglycaemia<sup>1,2</sup>. Unfortunately, islet allografts have been associated with only brief periods (4–8 d) of normoglycaemia, and attempts to prolong function with immunosuppressive therapy of the host have generally been unsuccessful<sup>3</sup>. The present results, however, suggest that the survival time of pancreatic islets, transplanted directly into the liver of a histoincompatible recipient, is prolonged by *in vitro* culture of the tissue before transplantation. Complete or partial control lasting up to more than 160 d was achieved in 70% of recipients. Nevertheless, allogeneic cultured transplants are less effective in equivalent quantities than isogeneic grafts, in reversing an experimental diabetic state in rats.

Evidence has accumulated that the immunogenicity of certain types of allografts is to some extent amenable to experimental manipulation<sup>4</sup>. Organ culture before transplantation may deplete the tissue of viable haematogenous elements and lymphoid cells<sup>5,6</sup> and/or diminish the concentration or availability of stimulatory antigens on the cell surface membranes<sup>7</sup>. In fact, as early as 1934, Stone *et al.*<sup>8</sup> attempted to adapt endocrine tissue in organ culture before transplantation. Other investigations with parathyroid, adrenal and pituitary organ-cultured transplants have shown that a growth period *in vitro* delays an adverse response of the host to the grafts<sup>9</sup>. Up to now, in spite of conflicting data about the enhancing effect of *in vitro* culture on the survival of skin grafts<sup>10</sup>, well controlled experiments have established that as a result of maintenance *in vitro* for 3 to 13 d, a significant proportion of allografts of ovarian and thyroid tissues are able to override major histocompatibility barriers<sup>11,12</sup>. Apart from one study mentioned in a preliminary abstract<sup>13</sup> no previous report deals with enhancement of survival of endocrine pancreas allografts after organ culture.

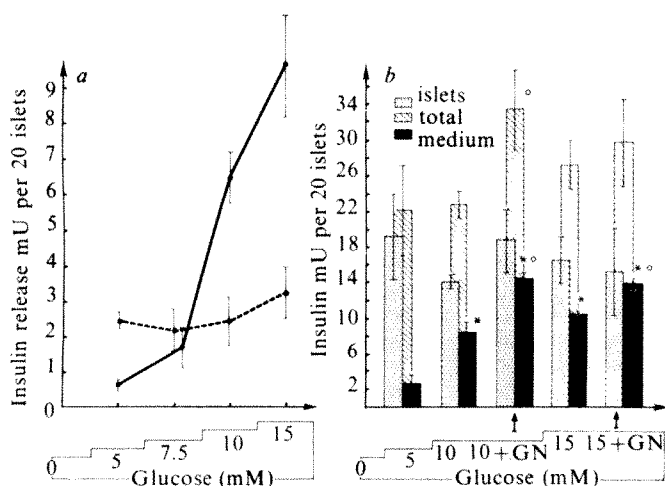
We have studied whether *in vitro* culture before transplantation enhances the survival of pancreatic endocrine allografts and have confirmed the functional value of cultured endocrine islets<sup>14</sup>. The endocrine pancreatic grafts

were transplanted directly into the anterior hepatic lobe of the recipient, since several investigators have suggested that the liver may exert a protective role on allograft tolerance (for reviews see refs 15–17). Diabetes was produced in young adult inbred male Lewis (LW) rats of 200 g body weight (Streptozotocin -IV- 65 mg kg<sup>-1</sup>). The rats received food and water *ad libitum* and each animal was isolated in a separate metabolic cage. Determination of body weight, daily measurements of urine volume and glucose content, and twice weekly measurements of serum glucose and insulin concentration were made. No animal was considered hyperglycaemic unless non-fasting blood glucose levels were over 4 g%, on the six determinations over a period of at least 15–18 d. Isolated endocrine islets from Wistar (WAG) inbred rats prepared by the classical collagenase digestion were collected under the dissecting microscope. One thousand islets, originating from three donors represented the amount of endocrine tissue grafted in each recipient. The islets were either transplanted immediately after their isolation or after culture for 4 or 5 d in Falcon dishes containing CMRL-1066 medium supplemented with 10% heat-inactivated calf serum<sup>14</sup>. Pancreatic transplantation of cultured or non-cultured endocrine islets was carried out by injecting the suspension of 1,000 islets in 0.5 ml of culture medium directly into the anterior hepatic lobe of the recipient. Neither exogenous insulin nor additional immunosuppressive therapy was administered to the recipients. The two inbred rat strains used were histoincompatible at the major locus (LW:Rt H-1<sup>i</sup> and WAG:H-1<sup>W</sup>) and this has been confirmed by cross skin grafts between donor and recipient strains.

As previously shown with mouse islets<sup>14</sup>, the culture of rat islets at 5 mM glucose concentration for 4 d led to a 50–60% decrease in the islet insulin content. The functional response of the cultured islets has been investigated (Fig. 1). Short term experiments (90 min) showed that 4-d cultured islets lost their sensitivity to glucose stimulation, compared with fresh islets. Insulin release by cultured islets, however, was stimulated by glucose alone or with glucagon, during prolonged incubation (24 h). Moreover, in these latter conditions the total insulin content (islets + medium) was always higher than that of the 4-d cultured islets (16.84 ± 3.2 mU per 20 islets), suggesting insulin synthesis by the cultured islets.

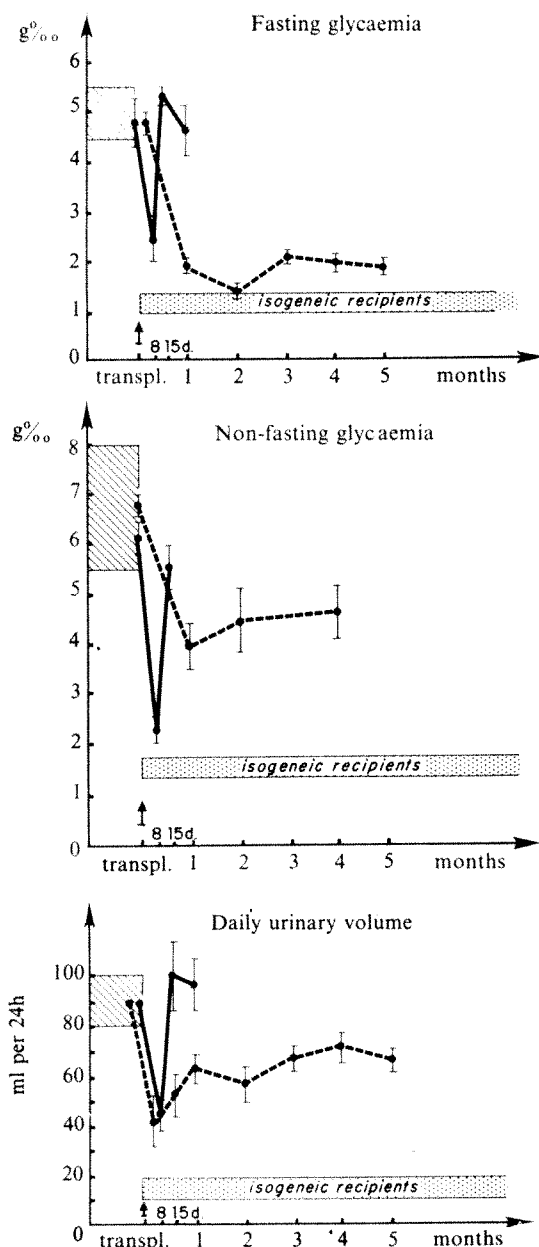
In animals treated with Streptozotocin (36 rats) the mean survival time (± s.e.m.) was about 25.5 ± 2.6 d following induction of the experimental diabetes. In animals receiving allogeneic non-cultured islet transplants (13 cases), the mean survival time was prolonged to 56.0 ± 7.3 d. Culture before transplantation increased the survival of the grafted animals significantly ( $P < 0.001$ ) (13 cases) to more than 161 d. Three of these animals are still surviving more than 6 months after the graft.

The biochemical and metabolic progress of the grafted animals has been compared with that we have observed previously<sup>2</sup> after isogeneic islet cell transplantation (Fig. 2). After allogeneic non-cultured islet transplantation, a temporary response was observed in 9 of 13 grafted animals. In the four remaining cases, the transplantation procedure did not significantly improve the diabetic state. This rate of success is similar to that observed previously in isogeneic islet transplantation<sup>2</sup>. The nine positive grafts, however, were followed by reversal to the diabetic state after a mean time of about 8.22 ± 0.81 d. On the other hand, among the 13 recipients of cultured allografts, 11 responded well to the graft, and only two of these reversed to the diabetic state between the 10th and 20th post-transplant day, while in the other nine a functioning graft survived for at least 90 d without immunosuppressive therapy of the host. In these animals the fasting level of blood sugar<sup>†</sup> was significantly improved as compared with pretransplant levels,



**Fig. 1** Glucose-stimulated insulin release of cultured islets. *a*, Short term incubation (90 min) of 4-d cultured islets (---) and fresh islets (—). Note the insensitivity of the cultured islets to increasing glucose concentrations. *b*, Prolonged incubation (24 h) of 4-d cultured islets in the presence of increasing glucose concentrations and addition of 10  $\mu$ g ml<sup>-1</sup> glucagon. Small non-significant variations of the insulin content of the islets are observed, whereas glucose (10 mM and 15 mM) significantly increased their insulin output. Further addition of glucagon potentiates the glucose effect. The results are expressed as mean values (mU per 20 islets ± s.e.m.) from 5 (*a*) to 10 (*b*) different experiments. Statistical significance was analysed by unpaired Student's *t* test; \*, *t* significant against 5 mM glucose ( $P < 0.001$ ); ○, *t* significant against glucose alone ( $P < 0.05$ ). GN, Glucagon.





**Fig. 2** Effect of transplantation of 1,000 islets on glycaemia and urinary volume of diabetic animals (shaded boxes) after: isogeneic islet transplantation (11 cases, dotted bar); non-cultured allogeneic islet transplantation (9 cases, —); 4-d cultured allogeneic islet transplantation (9 cases, — — —).

but remained significantly different from non-diabetic animals and/or isogeneic recipients. Moreover, the rise in blood sugar after feeding as well as urine volume were not controlled within normal limits. Insulinaemia of these animals ranged between 13 and 30  $\mu\text{U ml}^{-1}$  at the 5th post-operative month and was not different from that observed at the 8th post-transplant day, but was significantly different from pretransplant levels ( $P < 0.05$ ).

From these results we can say that *in vitro* culture before transplantation induces a fall in the insulin content of the islets. Allogeneic cultured transplants of endocrine tissue are functionally less effective in equivalent quantities than isogeneic grafts and are unable to control carbohydrate metabolism fully. Culture of the pancreatic islets, before transplantation, markedly enhances the host tolerance to the graft in the absence of immunosuppressive therapy and in spite of the existence of strong histoincompatible antigens between donor and recipient. Based on previous hypotheses and experimental results<sup>5,6</sup> the loss of metabolically active lymphoid cells during pancreatic islet

culture is now under investigation. The protective role of the liver on graft survival does not seem to be confirmed and seems to be limited to weak antigen barriers.

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1. Ballinger, W. F. & Lacy, P. E. *Surgery* 72, 175-186 (1972).
2. Eloy, R. *et al. Horm. metab. Res.* 9, 40-46 (1977).
3. Finch, D. R. A. & Morris, P. J. *Transplantation* 22, 503-512 (1976).
4. Billingham, R. E. *J. invest. Derm.* 67, 149-159 (1976).
5. Lafferty, K. J., Cooley, M. A., Woolnough, J. & Walker, K. Z. *Science* 188, 259-261 (1975).
6. Bach, F. H., Bach, M. L. & Sondel, P. M. *Nature* 259, 273-281 (1976).
7. Opelz, G. & Terasaki, P. I. *Science* 184, 464-466 (1974).
8. Stone, H. B., Owings, J. C. & Gey, G. O. *Ann. Surg.* 199, 613-626 (1934).
9. Gaillard, P. J. *Ciba Fdn Symp.* 100-106 (Churchill, London, 1964).
10. Ninnemann, J. L. & Good, R. A. *Transplantation* 18, 1-5 (1974).
11. Jacobs, B. B. *Transplantation* 18, 454-457 (1974).
12. Lafferty, K. J., Cooley, M. A., Woolnough, J. & Warler, K. Z. *Science* 188, 259-261 (1975).
13. Boyles, R. R. & Seltzer, H. S. *Diabetes (Abstr.)* 24, 420 (1975).
14. Kedinger, M., Moody, A. J., Launay, J. F. & Haffen, K. *Experientia* 33, 972-974 (1977).
15. Eloy, R., Vuitton, D., Vaultier, J. P., Pousse, A. & Grenier, J. F. *Cell. Immun.* 21, 236-242 (1975).
16. Vuitton, D., Eloy, R., Coumaros, G. & Grenier, J. F. *Cell. Immun.* 28, 51-58 (1977).
17. Vuitton, D., Eloy, R., Clendinnen, G. & Grenier, J. F. *Cell. Immun.* (in the press).

## Selective inhibition of response of *Ips pini* to its pheromone by the (S)-(—)-enantiomer of ipsenol

THE response of the bark beetle *Ips pini* (Say) (Coleoptera: Scolytidae) to the attractant produced by conspecific males boring in ponderosa pine is inhibited by the presence of boring males of *I. paraconfusus* Lanier<sup>1</sup>. Ipsenol, 2-methyl-6-methylene-7-octen-4-ol, one of three synergistic components required to elicit attraction in *I. paraconfusus*<sup>2</sup>, also reduces catches of *I. pini* at traps baited with boring male *I. pini*<sup>3,4</sup>. Ipsenol has not been found in *I. pini*. Because (S)-(—)-ipenol is the only enantiomer of ipsenol present in *I. paraconfusus*<sup>4</sup>, we compared the inhibitory activity of the enantiomers of ipsenol with that of the racemic compound in the laboratory and field and we found that *I. pini* is affected specifically by the (S)-(—)-enantiomer.

We measured the walking response of *I. pini* in an open-arena olfactometer<sup>5</sup> to aliquots of a cold-condensate of volatiles produced by 97 male *I. pini* while boring in ponderosa pine for 42 h (ref. 6). The attractant condensate was delivered in pentane from a power-driven syringe at about  $5.5 \times 10^{-2}$  beetle-minutes per min (one beetle-minute of condensate is equivalent to the attractant produced by one male boring for 1 min). The two enantiomers were synthesised from (S)-(+)-leucine and its antipode<sup>7</sup>, and both they and racemic ipsenol were delivered in pentane, by syringe, at  $5 \times 10^{-6} \mu\text{l min}^{-1}$ . Response was measured by the number of beetles reaching the source out of groups of ten released on the surface of the arena. All beetles used for assay were collected as they emerged in the laboratory from infested ponderosa pine and were stored in the same conditions before assays.

In the field, the response of *I. pini* was measured by the number trapped on cylindrical wire screen traps coated with a sticky material<sup>1,3</sup>. The attractive standard was produced by introducing 21 (test 1) and 25 (test 2) male beetles into pre-drilled holes in small ponderosa pine logs (15 × 30 cm). Each log was wrapped in fine metal screen to prevent attacks by responding beetles, and was placed inside the sticky trap on a pipe standard 1 m above ground. The compounds were evaporated from 5- $\mu$ l open-ended capillary tubes; one capillary suspended inside an inverted 35-mm film canister with a perforated lid. Two canisters were hung on opposite sides of a trap. The rates of evaporation of

**Table 1** Inhibition of the response of female *I. pini* to condensates of volatiles produced by boring male *I. pini*, by enantiomers of ipsenol

Test material	Response		No. of assays
	Mean of 10	(s.e.)	
Condensate	5.07	(2.94) (a)	6
Condensate + (R)-(+)-ipsenol	2.62	(1.98) (b)	13
Condensate + (S)-(-)-ipsenol	0.83	(1.03) (c)	12
Condensate + racemic ipsenol	0.17	(0.41) (c)	15
No stimulus	0.17	(0.41) (c)	6

Tests were conducted between 1 and 13 September, 1976 in Davis, California. Responses followed by different letters are significantly different at  $P < 0.05$  (Mann-Whitney test).

both enantiomers and racemic ipsenol, measured volumetrically in the field, were approximately 2 mg for 24 h for each treatment. Treatments were arranged in a line, 20 m apart. Beetles were picked from the traps which were rotated every 2 h, so that all treatments occupied all positions at least once.

The responses of female *I. pini* in the laboratory to cold condensate alone and to condensate delivered with (R)-(+)-ipsenol were both significantly higher than those to condensate delivered with either racemic ipsenol or (S)-(-)-ipsenol and to the control (no condensate or ipsenol) (Table 1). The slight, but significant, activity of (R)-(+)-ipsenol in inhibiting female response to cold condensate could easily have been due to contamination, which is difficult to avoid in that type of assay. Too few males were available to provide a significant result, but their response paralleled that of the females.

In field tests, (R)-(+)-ipsenol had no measurable effect on catches of *I. pini* at traps containing males boring in logs (Table 2). In contrast, on traps where racemic or (S)-(-)-ipsenol was evaporated beside a log containing males, there

**Table 2** Effect of enantiomers and racemic ipsenol on catches of *I. pini* at traps containing male *I. pini* boring in ponderosa pine

Treatment	Test 1			Test 2		
	Total	Median	$\delta:\phi$	Total	Median	$\delta:\phi$
<i>I. pini</i>	47	3(a)	1:1.8	32	2(a)	1:0.8
<i>I. pini</i> + (R)-(+)-ipsenol	27	3(a)	1:1.25	43	3(a)	1:1.26
<i>I. pini</i> + (S)-(-)-ipsenol	1	0(b)	—	2	0(b)	0:2.0
<i>I. pini</i> + racemic ipsenol	8	0(b)	—	3	0(b)	3:0.0
Ponderosa pine log alone	0	0	—	3	0(b)	—
Racemic ipsenol alone	0	0	—	0	0	—

Test 1 was conducted between 6 and 9 July and test 2 between 17 and 21 July, 1977 at McCloud Flats, California. Eleven 2-h replications of each treatment were done in each test. There were 21 males per bolt in test 1 and 25 in test 2. Trap catches followed by different letters are significantly different (Wilcoxon signed rank test) at  $P < 0.01$  (test 1) and  $P < 0.05$  (test 2).

were so few catches that there was no significant difference from control traps containing either a log with no beetles or racemic ipsenol alone. Thus, the walking responses of *I. pini* measured in the laboratory paralleled the response in the field, although with less precision. From both sets of results we conclude that the inhibition of aggregation in *I. pini* by boring males of *I. paraconfusus* is probably due to (S)-(-)-ipsenol released by those males.

Although enantiomeric specificity of the components of attractant pheromones has been demonstrated in several species of Scolytidae, *Dendroctonus*<sup>8</sup>, *Gnathotrichus*<sup>9</sup>, and *Ips*<sup>10-12</sup>, we believe that ours is the first demonstration of enantiomeric specificity of an interspecific pheromonal inhibitor in this family. The restricted production of and response to enantiomers is one mechanism which enhances the range of unique combinations of available olfactory stimuli.

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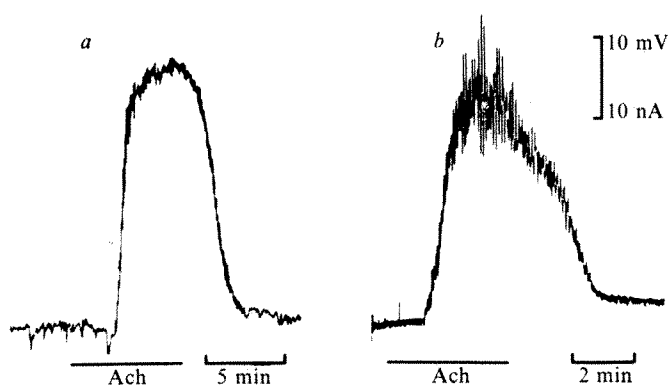
Received 26 July; accepted 28 October 1977.

1. Birch, M. C. & Wood, D. L. *J. chem. Ecol.* **1**, 101-113 (1975).
2. Silverstein, R. M., Rodin, J. O. & Wood, D. L. *Science* **154**, 509-510 (1966).
3. Birch, M. C. & Light, D. M. *J. chem. Ecol.* **3**, 257-267 (1977).
4. Mori, K. *Tetrahedron Lett.* **26**, 2187-2190 (1975).
5. Wood, D. L., Browne, L. E., Silverstein, R. M. & Rodin, J. O. *J. Insect Physiol.* **12**, 523-536 (1966).
6. Browne, L. E., Birch, M. C. & Wood, D. L. *J. Insect Physiol.* **20**, 183-193 (1974).
7. Mori, K. *Tetrahedron*, **32**, 1101-1106 (1976).
8. Wood, D. L. *et al. Science* **192**, 896-898 (1976).
9. Borden, J. H., Chong, L., McLean, J. A., Slessor, K. N. & Mori, K. *Science* **192**, 894-896 (1976).
10. Vité, J. P., Klimetzek, D., Loskant, G., Hedden, R. & Mori, K. *Naturwissenschaften* **63**, 582-583 (1976).
11. Vité, J. P., Hedden, R. & Mori, K. *Naturwissenschaften* **63**, 43 (1976).
12. Hedden, R., Vité, J. P. & Mori, K. *Nature* **261**, 696-697 (1976).

## Acetylcholine receptors in the oocyte membrane

In vertebrates, fully differentiated cells such as skeletal muscle and some nerve cells are highly sensitive to acetylcholine (ACh), due to the presence of acetylcholine receptors in their surface membranes. In muscle fibres, the ACh-sensitivity is known to appear early during differentiation<sup>1-3</sup>, but relatively little is known about the chemical sensitivity of the membrane in the undifferentiated cell. In particular, it would be interesting to know if spermatozoa and oocytes are already sensitive to ACh and other neurotransmitter substances. To examine this question, toad (*Xenopus laevis*) oocytes were taken from the ovary, kept in frog's Ringer or Merriam<sup>4</sup> solution, and studied at room temperature, using conventional electrophysiological techniques.

The oocytes had resting potentials of -30 to -80 mV, and their input resistance varied between 200 k $\Omega$  and over 3 M $\Omega$ . The membrane potential frequently showed transient spontaneous depolarisations a few mV in amplitude. When the oocytes were perfused with a solution containing ACh the membrane was depolarised as illustrated in Fig. 1a. This depolarisation was dose-dependent; but varied greatly among different oocytes, with some responding to as little as 10<sup>-8</sup> M, while oocytes from other animals failed to respond to 10<sup>-3</sup> M. Other cholinergic drugs such as carbachol, muscarine, arecholine and choline also depolarised the oocytes. The most usual response to ACh was a membrane depolarisation but frequently this was followed, or preceded, by a hyperpolarisation; and in some oocytes, only a hyperpolarising response was seen. Both types of response



**Fig. 1** Effect of acetylcholine (ACh) on a *Xenopus laevis* oocyte. *a*, Change in membrane potential induced by bath application of  $10^{-5}$  M ACh. The bar indicates the time during which ACh was perfused. Resting potential  $-63$  mV. *b*, Membrane current induced by perfusion of  $10^{-5}$  M ACh. Same oocyte as in *a* but under voltage-clamp. Holding potential  $-53$  mV. Oocyte pre-treated with collagenase to remove follicular cells.

'desensitised' during maintained application of ACh. To determine if the ACh was acting directly on the oocyte membrane, and not through an action on the follicular cells which normally envelop the oocytes, these were treated with collagenase which is known<sup>5</sup> to remove the follicular cells. Such treatment sometimes reduced or even abolished the effect of ACh, but many treated oocytes responded normally to ACh and a subsequent morphological study showed that the follicular cells had actually been removed. Thus, it is clear that ACh is acting directly on the oocyte.

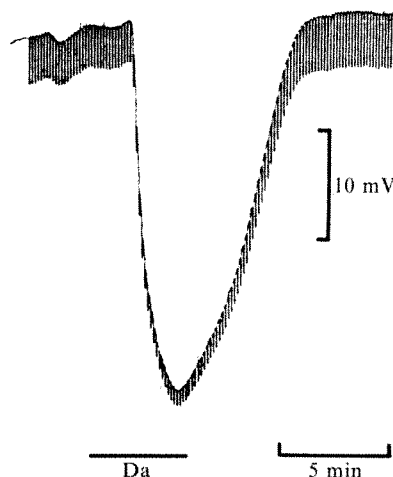
The depolarisation induced by ACh was accompanied by a fall in membrane resistance (Fig. 2), and an inward flux of current which could be measured under voltage-clamp conditions (Fig. 1*b*). The membrane potential at which the ACh-induced current reverses in direction was about  $-25$  mV, and similar values were obtained from the cross-over point of the current-voltage relations before and during the application of ACh (Fig. 2) or from the plateau level of depolarisation evoked by high doses of ACh (Fig. 1*a*).

In other systems, ACh is known to alter membrane permeability to one or more of the following ions:  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and possibly  $\text{Ca}^{2+}$  (ref. 6). ACh was still able to depolarise the oocytes when  $\text{Ca}^{2+}$  was removed from the

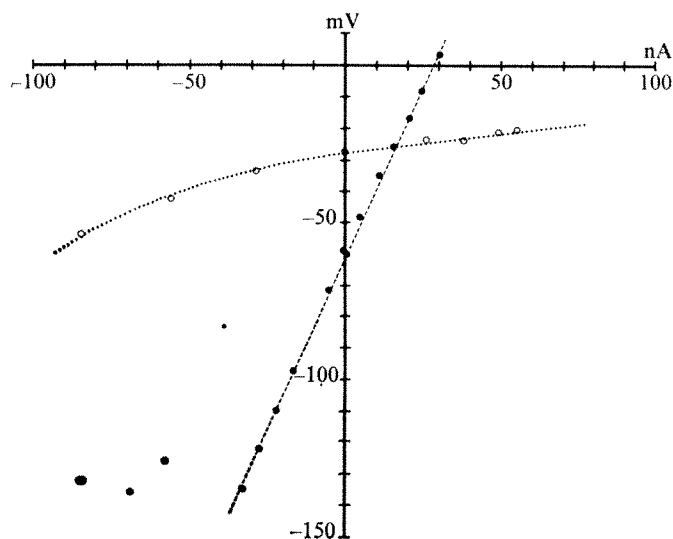
external fluid, when Tris,  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  were completely substituted for  $\text{Na}^+$ , or when all the  $\text{Cl}^-$  was replaced by  $\text{SO}_4^{2-}$ . In the latter case, the response to ACh was enhanced and the membrane potential during ACh-action even reversed in sign, becoming inside positive. The principal conclusion drawn from these experiments is that ACh increases the permeability of the oocyte membrane, mainly to  $\text{Cl}^-$  ions.

Some substances which are known to block the effects of ACh in other systems were tested to see if they antagonised the action of ACh on the oocyte. Curare ( $10^{-6}$  to  $10^{-4}$  M) was ineffective, as was also  $\alpha$ -bungarotoxin ( $10^{-6}$  g  $\text{ml}^{-1}$ ) which did not block the response to ACh even after the oocytes had been incubated in the toxin for 48 h. Tetrodotoxin ( $10^{-6}$  M), which blocks action potentials in some cells, did not abolish the spontaneous membrane depolarisations or the effect of ACh. On the other hand, atropine ( $10^{-7}$  to  $10^{-4}$  M) blocked the action of ACh on the oocytes in a reversible way.

We have made a brief survey of the effect of some neurotransmitters on the oocytes, by perfusing the drugs at concentrations ranging from  $10^{-6}$  to  $10^{-3}$  M. Glycine, GABA, *L*-glutamate and histamine had no obvious effect on the membrane potential of collagenase-treated, or untreated oocytes. Adrenaline, 5-hydroxytryptamine and dopamine evoked a membrane hyperpolarisation accompanied by a decrease in membrane resistance (Fig. 3). As in the case of ACh, the sensitivity of the oocytes to the amines varied considerably from batch to batch. Some oocytes were sensitive to both ACh and dopamine, in which case atropine blocked only the response to ACh. Thus, ACh and dopamine seem to act on different membrane receptors, and evoke different permeability changes.



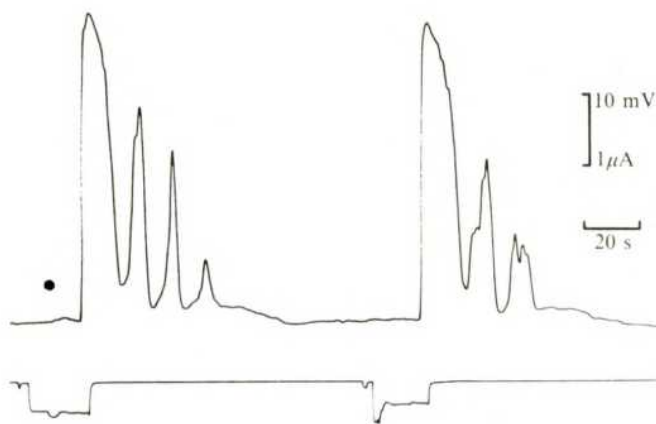
**Fig. 3** Hyperpolarisation of oocyte membrane potential induced by dopamine ( $10^{-6}$  M). Membrane resistance decreased during dopamine action, as evidenced by the decrease in the potential change caused by the series of brief hyperpolarising pulses. Oocyte not pre-treated with collagenase. Resting potential,  $-41$  mV.



**Fig. 2** Current-voltage relation of the oocyte membrane in resting state (full circles) and during depolarisation induced by  $10^{-5}$  M ACh ( $\circ$ ). Membrane conductance increased about 18-fold during peak ACh-action. Collagenase-treated oocyte.

During bath application of ACh (Fig. 1), it was noticed that the response began well after the 'dead-time' of the perfusion system (about 1 min). To obtain a better indication of the time lag between the application of ACh and the onset of ACh-action, we applied the ACh ionophoretically to a small patch of the oocyte's surface membrane. Using this method on muscle fibres it has been possible<sup>7</sup> to detect a response to ACh a small fraction of a millisecond after the release of ACh. In contrast, the responses in the oocyte always took many seconds to develop (Fig. 4) and consisted of a series of oscillations of membrane potential. Similar





**Fig. 4** Oscillations of membrane potential induced by iontophoretic application of ACh to an oocyte pre-treated with collagenase. Upper trace: membrane potential. Lower trace: ionophoretic current used to release ACh. The small deflexion preceding each pulse gives a measure of the backing current used to retain the ACh in the pipette. Resting membrane potential,  $-62$  mV.

oscillations in current were seen when the oocyte was in voltage-clamp (cf. Fig. 1b).

Micro-iontophoresis of ACh also allowed us to examine the distribution of ACh-sensitivity over the oocyte's surface. There was a large variation in the sensitivity of different spots, but nevertheless it was clear that both the pigmented and non-pigmented surfaces of the oocyte were sensitive to ACh. Furthermore, it seems that the ACh-receptors are only activated by ACh from the outside, because no depolarisation was obtained when the ACh was applied intracellularly.

Thus, it seems that the oocytes synthesise, and incorporate into their surface membrane, receptors which are activated by neurotransmitters and related substances. The functional significance of these receptors in the oocyte is not known, although it has been suggested that ACh and some monoamines may act as intracellular regulators of cell division<sup>8,9</sup>.

Our experiments indicate that the drug-receptor combinations open channels through which ions flow across the oocyte membrane. The long delay between the application of ACh and the onset of the increase in membrane permeability indicates that the receptor-channel coupling is not as fast as in skeletal muscle. Perhaps a number of receptors have to interact before they are able to open the channel; or it may be that in the oocyte the opening of the channel is not triggered directly by the receptor, but indirectly through the production of a substance, perhaps within the cell, and its accumulation near the membrane.

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1. Diamond, J. & Miledi, R. *J. Physiol., Lond.* **162**, 393–408 (1962).
2. Ritchie, A. K. & Fambrough, D. M. *J. gen. Physiol.* **65**, 327–356 (1975).
3. Blackshaw, S. & Warner, A. *Nature* **262**, 217–218 (1976).
4. Merriam, R. W. *Expl Cell Res.* **68**, 81 (1971).
5. Moreau, M., Guerrier, P. & Dorée, M. *C. r. hebdom. Séanc. Acad. Sci. Paris* **D282**, 1309–1312 (1976).
6. Ginsborg, B. L. *Biochim. Biophys. Acta* **300**, 289–317 (1973).
7. Katz, B. & Miledi, R. *Proc. R. Soc. Lond. B* **161**, 483–495 (1965).
8. Reshetnikova, N. A. *Zh. Evol. Biochim. I. Fiziol.* **6**, 19–24 (1970).
9. Buznikov, G. A. *Ontogenes* **2**, 5–13 (1971).

## Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones

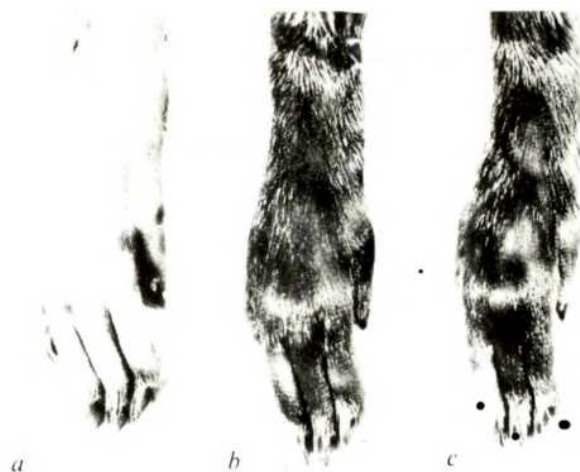
PHARMACOLOGICALLY evoked selective degeneration of neurones with a specific function or functioning with known transmitter substances, has been reported only in the monoaminergic neurone systems<sup>1,2</sup>. We report here that selective degeneration of neurones with a highly specific function can be produced by chemical agents in the somatosensory system as well. Capsaicin given to newborn rats induces selective degeneration of a distinct population of primary sensory neurones involved in mediation of chemogenic pain.

Capsaicin—after an initial violent stimulation—renders sensory nerve endings in the skin and mucous membranes of different species insensitive to chemical pain stimuli for a long time<sup>3,4</sup>. In addition, in the rat, marked systemic desensitisation greatly inhibits the neurogenic inflammation induced either by pain-producing chemical irritants, or by antidromic electrical stimulation of sensory nerves<sup>5,6</sup>. The neurohumoral substance which increases vascular permeability is released from the sensory nerve endings which transmit chemogenic pain<sup>7–8</sup>. Thus the degree of the increase in vascular permeability can be used as a tool to study the functional condition of primary sensory neurones involved in the transmission of pain induced by chemical stimuli. The desensitising effect of capsaicin has been analysed in detail in the adult rat, and it has been established that the functional impairment ensues at the level of the primary sensory neurone<sup>6,9,10</sup> without causing its degeneration<sup>11</sup>.

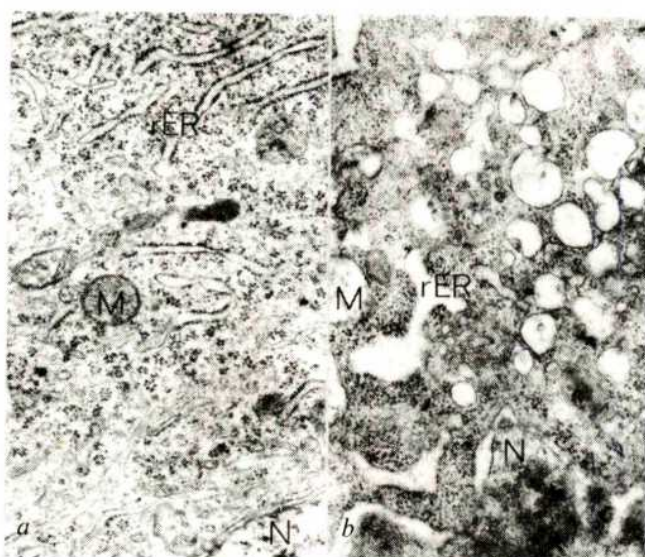
Newborn rats of the CFY strain were given a moderate subcutaneous dose of  $50 \text{ mg kg}^{-1}$  capsaicin on day 2 of life. Animals receiving a similar amount of the solvent used (10% ethanol, 10% Tween 80 in isotonic saline) served as controls. Adult rats, aged 3–4 months, were similarly treated. Neurogenic inflammation was brought about by painting the skin of one of the hind paws with 5% mustard oil in liquid paraffin or with xylene<sup>5,6</sup> after intravenous administration of  $50 \text{ mg kg}^{-1}$  Evans Blue dye. Twenty minutes afterwards, the animals were bled and the amount of exuded dye was extracted from the skin with methanol containing 1% Suramin and determined photometrically<sup>12</sup>.

Table 1 shows the results of a series of experiments

**Fig. 1** Effect of 5% mustard oil on the skin of the paw of a rat pretreated *a*, neonatally and *b*, as an adult with  $50 \text{ mg kg}^{-1}$  capsaicin 9 months before the experiment; *c*, normal control. Evans Blue dose was  $50 \text{ mg kg}^{-1}$  intravenously.







**Fig. 2** Electron micrograph of B-type neurones of cervical sensory ganglia of 2-d-old rats. *a*, Control; *b*, 30 min after capsaicin treatment. As a consequence of capsaicin treatment, the fine structure of the primary sensory neurone is severely impaired; note swollen mitochondria, disorganisation of cristae, dilation of the perinuclear cisterna, as well as that of the cisternae of the rough endoplasmic reticulum. The electron density of the cytoplasm much greater than that of the control. N, Nucleus; M, mitochondrium; rER, cisterna of the rough endoplasmic reticulum. Magnification  $\times 16,000$ .

carried out on rats pretreated, as neonates or as adults, with capsaicin 2–8 months before the experiment. The amount of dye exuded from rats pretreated as adults did not differ considerably from that of the controls. Neonatal pretreatment, however, resulted in an almost complete blockade of the neurogenic inflammatory response. Figure 1 shows the results of a typical experiment, where pretreatment was  $50 \text{ mg kg}^{-1}$  capsaicin 9 months before eliciting neurogenic inflammation in neonatal and adult rats. The paw of the control rat turned blue in response to 5% mustard oil solution, indicating the intense increase in the vascular permeability. A comparable dye exudation also ensued in the skin of a rat pretreated as an adult. Neurogenic inflammation could not, however, be elicited in the paw of the neonatally-pretreated rat. The same results were obtained if xylene was used as the irritant. Chemical sensitivity in the eye, as tested by zingerone, the pungent pain-producing substance in ginger<sup>5</sup>, was practically abolished in these animals, but they showed no reduction in sensitivity to physical, for example, mechanical, stimuli. Neonatal capsaicin pretreatment evidently impairs the function of the chemosensitive primary sensory neurones irreversibly.

For histological investigations, animals were perfused with a buffered solution of 2% glutaraldehyde and 4% formaldehyde by way of the left ventricle, at different times

after neonatal capsaicin pretreatment. The Gasserian ganglion, cervical sensory ganglia and attached dorsal roots and the cervical spinal cord were processed for standard electron microscopic examination. In agreement with previous reports, the two principal types of sensory ganglion cells, the large type A neurones and the small type B neurones<sup>13</sup> could be distinguished even at the very early stage of postnatal development<sup>14</sup>. Capsaicin injected to 2-d-old rats exclusively damaged some of the B-type neurones. Only 30 min after administration of capsaicin, these neurones showed severe fine structural alterations (Fig. 2). The ultrastructure of the type A neurones and other cellular elements of sensory ganglia was unimpaired after capsaicin treatment. Similarly, the fine structure of all cellular elements of sensory ganglia obtained from control animals appeared normal.



**Fig. 3** Electron micrograph showing degenerated axons and axon terminals (arrows) partially engulfed by glial processes (double arrows), in Rexed's lamina II of the cervical spinal cord 8 h after capsaicin administration to a 2-d-old animal. Inset: Degenerated axon terminal still in synaptic contact with a postsynaptic dendrite. Magnification:  $\times 5,920$ , inset  $\times 13,500$ .

Light microscopy of 3–6- $\mu\text{m}$  thick, *p*-phenylenediamine-stained<sup>15</sup> Araldite sections of the cervical spinal cord of neonatally-treated rats which had been killed 8 h after treatment, revealed degeneration of axon terminals in the head of the dorsal horn, mainly in the laminae I and II of Rexed. Electron microscopy confirmed this finding by showing the presence of a considerable number of degenerating axons and axon terminals in this area (Fig. 3). After survival for 15 h, most of the degenerated axon terminals were engulfed by glial processes. The degenerating axon terminals are, in all probability, the central processes of the impaired B-type sensory ganglion cells, since 4 h after capsaicin treatment many unmyelinated nerve fibres in the

**Table 1** Evans Blue dye exudation elicited by 5% mustard oil in the paw skin of rats pretreated with capsaicin 2–8 months before the experiment

Rats (No.)	Pretreatment	Excess dye ( $\mu\text{g}$ ) ( $\pm\text{SE}$ )	Inhibition (%)
17	—	23.75 $\pm$ 4.03	
17	50 mg $\text{kg}^{-1}$ capsaicin (neonatally)	1.48 $\pm$ 0.5	93.8
10	—	27.78 $\pm$ 6.18	
10	50 mg $\text{kg}^{-1}$ capsaicin (adult)	19.05 $\pm$ 6.45	31.5

The excess dye values were obtained by subtracting the dye content of the control skin from that of the treated skin. Evans Blue dose 50 mg  $\text{kg}^{-1}$  intravenously.

dorsal root underwent degeneration. In addition, experiments now in progress show, that one month after neonatal capsaicin treatment there is about a 70% reduction in the number of unmyelinated fibres of the saphenous nerve.

Axonal degeneration in the dorsal horn was accompanied by the appearance of many glial cells, localised mainly in the laminae I and II of Rexed. In stained sections, the cytoplasm of these cells contained intensely-stained granules and they were also characterised by a very marked acid phosphatase enzyme activity. In the electron microscope sections, numerous lamellar bodies and osmiophil debris were observed in the cytoplasm of these cells. It therefore seems that these cells are phagocytosing glial cells, ingesting degenerated neuronal elements.

The findings presented here clearly show that neonatal capsaicin treatment results in a completely irreversible impairment of the function of chemosensitive primary sensory neurones. As a consequence, painful sensory irritants, the effect of which depends on intact chemosensitive nerve endings, do not evoke neurogenic inflammation in neonatally-treated animals. The morphological basis for this phenomenon is provided by the degeneration of a distinct population of primary sensory neurones. In adult rats treated with capsaicin, the effect of neurogenic irritants gradually reappeared, showing that the function of chemosensitive pain nerve endings, at least partially, returned. This latter finding is consistent with previous results, which stated that capsaicin treatment does not induce degeneration of primary sensory neurones in the adult rat<sup>11</sup>.

Our present results may open new possibilities in the study of the structure and function of chemosensitive nerve endings, as well as in mapping of their central neuronal connections. These findings may also promote investigations to elucidate, for example, immunohistochemically, which of the putative transmitters<sup>16</sup> may be involved in mediation of chemogenic pain.

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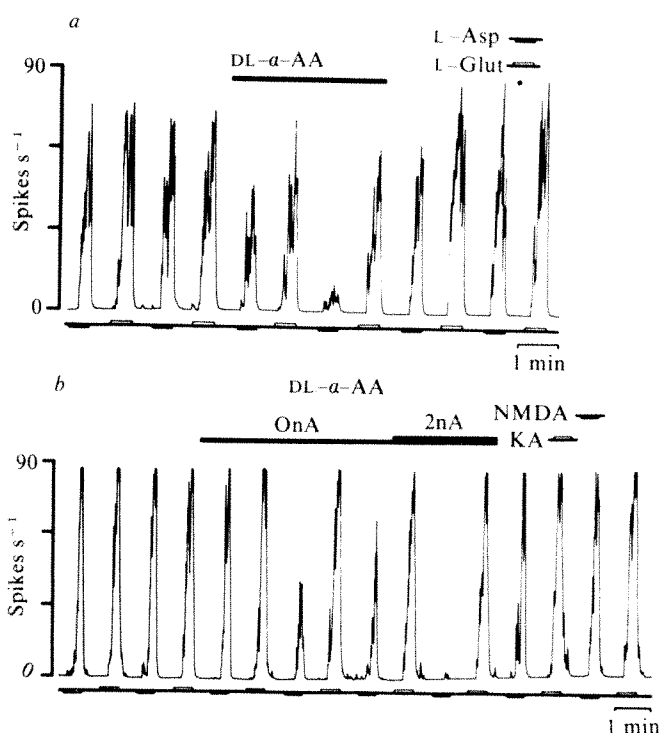
Received 21 June; accepted 25 October 1977.

- Kostrzewa, R. M. & Jacobowitz, D. M. *Pharmac. Rev.* **26**, 199–288 (1974).
- Baumgarten, H. G., Björklund, A., Nobin, A., Rosengren, E. & Schlossberger, H. G. *Acta physiol. scand., Suppl.* **429**, 7–29 (1975).
- Jancsó, N. *Bull. Millard Fillmore Hosp. Buffalo N.Y.* **7**, 53–77 (1960).
- Jancsó, N. & Jancsó-Gábor, A. *Arch. exp. Path. Pharmac.* **236**, 142–145 (1959).
- Jancsó, N. *Proc. 3rd Int. Pharmac. Mtg, 1966. Pharmacology of Pain* **9**, 33–55 (Pergamon, Oxford, New York, 1968).
- Jancsó, N., Jancsó-Gábor, A. & Szolcsányi, J. *Br. J. Pharmac.* **31**, 138–151 (1967).
- Jancsó-Gábor, A. & Szolcsányi, J. *J. dent. Res.* **51**, 264–269 (1972).
- García Leme, J. & Hamamura, L. *Br. J. Pharmac.* **51**, 383–389 (1974).
- Pórszász, J. & Jancsó, N. *Acta physiol. Acad. Sci. Hung.* **16**, 299–306 (1959).
- Jancsó, G. & Knyihár, E. *Neurobiology* **5**, 42–43 (1975).
- Joó, F., Szolcsányi, J. & Jancsó-Gábor, A. *Life Sci.* **8**, 621–626 (1969).
- Jancsó-Gábor, A., Szolcsányi, J. & Jancsó, N. *J. Pharm. Pharmac.* **19**, 486–487 (1967).
- Andres, K. H. Z. *Zellforsch.* **55**, 1–48 (1961).
- Yamadori, T. *Acta Anat. Nippon.* **45**, 191–205 (1970).
- Holländer, H. & Vaaland, J. L. *Brain Res.* **10**, 120–126 (1968).
- Hökfelt, T. *et al. Neuroscience* **1**, 131–136 (1976).

## D- $\alpha$ -Aminoadipate as a selective antagonist of amino acid-induced and synaptic excitation of mammalian spinal neurones

THE identification of either L-glutamate or L-aspartate as excitatory transmitters in the mammalian central nervous system would be facilitated by the discovery of specific antagonists of amino acid-induced and synaptic excitation. Hall *et al.* have suggested that D- $\alpha$ -aminoadipate may be an amino acid antagonist<sup>1</sup>. They based their suggestion on the

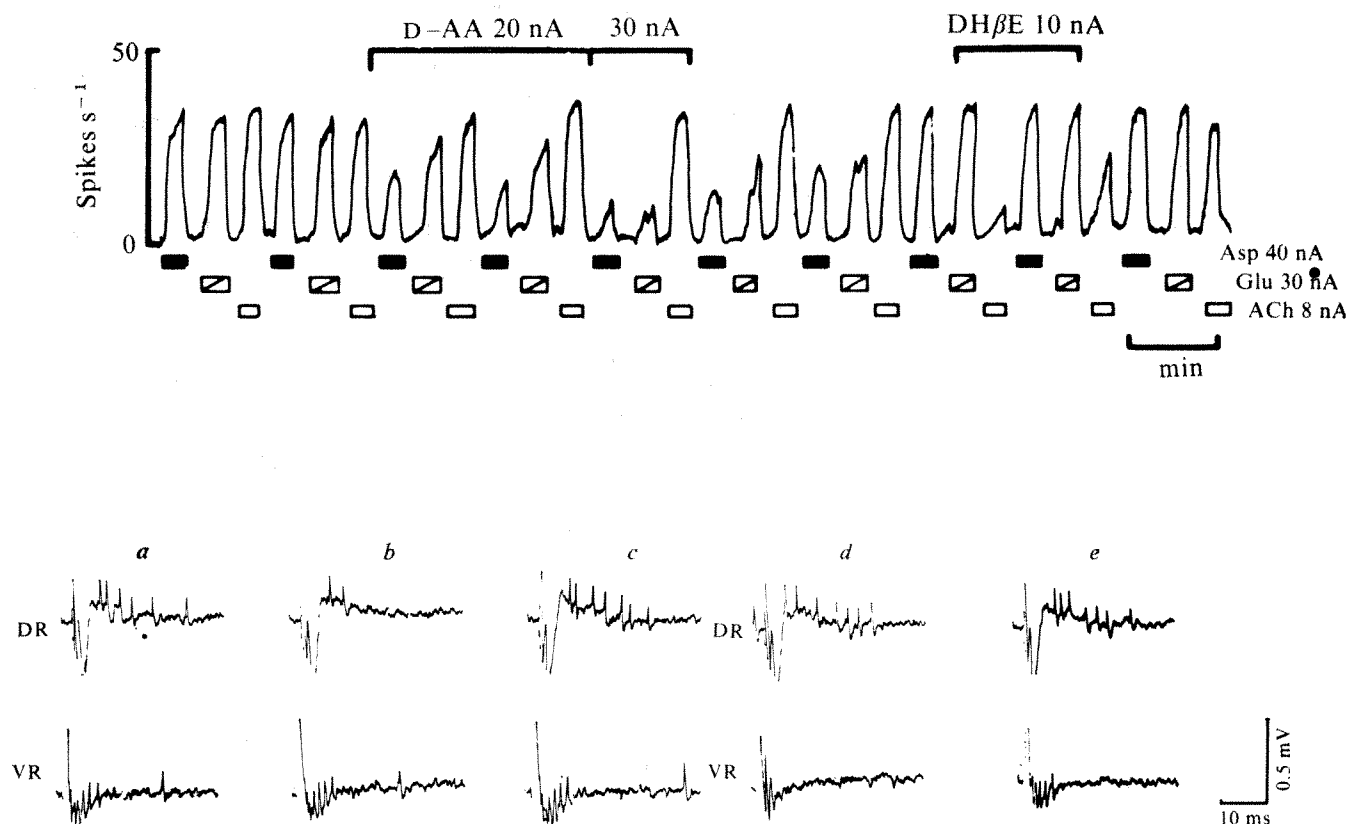
depressant effect of the DL form on spontaneous and amino acid-induced excitation of rat thalamic neurones, the L form being weakly excitatory. We have shown that related long-chain amino acids depress synaptic excitation of spinal neurones and exert a differential depressant action on chemically induced excitation of these cells<sup>2</sup>. We therefore tested the actions of both D- and DL- $\alpha$ -aminoadipate on synaptically evoked excitation and on excitations induced by acetylcholine, L-glutamate, L-aspartate, kainate and N-methyl-D-aspartate (NMDA) on spinal neurones. These latter two amino acids have been suggested to act on 'glutamate-preferring' and 'aspartate-preferring' receptors, respectively<sup>3,4</sup>. The results presented here indicate that D- $\alpha$ -aminoadipate selectively antagonises NMDA- and L-aspartate-induced responses of dorsal horn interneurones and Renshaw cells and depresses non-cholinergic synaptic excitation of these cells evoked by dorsal root stimulation, whereas cholinergic excitation of Renshaw cells evoked by iontophoretic acetylcholine or ventral root stimulation is not depressed by this agent.



**Fig. 1** Selective effects of DL- $\alpha$ -aminoadipate (DL-AA) on amino acid-induced excitation of a dorsal horn interneurone in the mouse spinal cord. *a*, DL-AA (6 nA), ejected for the period indicated by the upper bar, almost abolished excitation produced by L-aspartate (30 nA) and depressed responses of the cell to L-glutamate (38 nA) to a lesser extent. *b*, Responses of the same cell to NMDA (24 nA) were diminished by removal of the DL-AA retaining current and abolished by 2 nA of the agent, while responses to kainate (5 nA) were unaffected.

We used spinal interneurones of the mouse and spinal interneurones and Renshaw cells of the cat. Mice (C3H strain) were anaesthetised with pentobarbitone sodium (40 mg per kg body weight) given periodically through an intraperitoneal (i.p.) cannula as required. After i.p. administration of gallamine triethiodide (50 mg per kg), mice were ventilated artificially through a tracheal cannula at a respiratory rate of 166 min<sup>-1</sup>. They rested on a thermistor-controlled heating pad (37–38 °C). (Further details are given in ref. 5.) Cats were anaesthetised with pentobarbitone sodium (35 mg per kg i.p. initially, supplemented by 5 mg per kg i.v. when necessary). The surgical procedure and methods of neurone identification were as previously described<sup>6</sup>. Conventional iontophoretic techniques were used in both series of experiments. Extracellular recordings





**Fig. 2** Selective antagonist actions of D- $\alpha$ -aminoadipate (D-AA) and dihydro- $\beta$ -erythroidine (DH $\beta$ E) on chemically- and synaptically-induced firing of a cat Renshaw cell. The ratemeter record at the top of the figure illustrates the selective depression of L-glutamate (glu 30 nA) and L-aspartate (asp 40 nA)-induced excitation by D-AA 20–30 nA, and of acetylcholine (ACh 8 nA)-induced excitation by DH $\beta$ E (10 nA). Records *a–e* are representative oscilloscope sweeps of the synaptic responses of the same Renshaw cell to a constant submaximal dorsal root (DR) and ventral root (VR) stimulus. *a* was taken during the control period, *b* during the ejection of D-AA 90 nA for 20 min, *c* 2 min after terminating the ejection of D-AA, *d* during the ejection of DH $\beta$ E 50 nA for 2 min, and *e* 3 min after terminating the DH $\beta$ E ejection. The mean number of spikes in 15 responses  $\pm$  s.e.m. were, for upper and lower traces respectively, *a*,  $5.8 \pm 0.7$  and  $4.1 \pm 0.2$ ; *b*,  $1.8 \pm 0.1$  and  $4.7 \pm 0.3$ ; *c*,  $5.3 \pm 0.7$  and  $4.8 \pm 0.2$ ; *d*,  $4.9 \pm 0.6$  and  $2.3 \pm 0.3$ ; *e*,  $4.7 \pm 0.8$  and  $3.6 \pm 0.3$ . D-AA reversibly reduced the DR responses but not the VR responses (*b*), while DH $\beta$ E reversibly reduced the VR responses but not the DR responses (*d*). These specific depressant effects of D-AA and DH $\beta$ E are highly significant statistically ( $P < 0.001$  in both cases, Student's *t* test).

were made with the 4 M NaCl-containing centre barrel of seven-barrel micropipettes, the other barrels containing solutions of agonists and antagonists as follows: sodium NMDA, 50 mM in 100 mM NaCl, pH 7; sodium kainate, 20 mM in 130 mM NaCl, pH 7; sodium L-glutamate and sodium L-aspartate, each 200 mM (mouse) or 500 mM (cat), pH 7; sodium D- and sodium DL- $\alpha$ -aminoadipate (Sigma) each 200 mM, pH 7; acetylcholine chloride (500 mM). D- $\alpha$ -Aminoadipic acid ( $[\alpha]_D^{25} -23.5^\circ$ ,  $c$  0.7 in 6 N HCl) was isolated from the racemic mixture by fractional crystallisation of the D-lysine salt, and recovery of the free acid by ion exchange chromatography.

Both D- and DL- $\alpha$ -aminoadipate selectively depressed NMDA- and L-aspartate-induced excitation of dorsal horn interneurons in the mouse spinal cord. The cell illustrated in Fig. 1 was particularly sensitive to the antagonist and an ejection current of 2 nA was sufficient to abolish the excitation produced by NMDA. There was little or no effect on excitation evoked by kainate (Fig. 1*b*). A slightly higher antagonist-ejecting current (6 nA) almost abolished L-aspartate-induced excitation of the same cell, and had considerably less effect on L-glutamate-induced excitation (Fig. 1*a*). Recovery of the responses occurred rapidly after termination of the antagonist administration. Similar effects were seen on all 14 cells treated with either D- $\alpha$ -aminoadipate ( $11 \pm 2$  nA, 7 cells) or DL- $\alpha$ -aminoadipate ( $17 \pm 6$  nA, 7 cells). Both forms of  $\alpha$ -aminoadipate also depressed the synaptic excitation of these cells evoked by dorsal root stimulation. This effect was seen on 8 of 10 cells tested with the D form (20–45 nA) and on all four cells tested with the DL form (5–40 nA).

When tested on cat Renshaw cells, both DL- $\alpha$ -aminoadipate ( $39 \pm 12$  nA, six cells) and D- $\alpha$ -aminoadipate ( $16 \pm 4$  nA, 10 cells) had a similar differential depressant effect on excitation induced by NMDA and kainate to that observed on mouse dorsal horn interneurons. L-Aspartate-induced excitation was also more sensitive than L-glutamate-induced excitation of these cells, though this differential sensitivity tended to diminish with increased ejection currents of antagonist (Fig. 2). Acetylcholine-induced excitation of the same Renshaw cells was virtually unaffected by either form of the agent. Of 16 Renshaw cells excited by acetylcholine, 13 were unaffected, 1 depressed and 2 slightly enhanced by DL- or D- $\alpha$ -aminoadipate (6 and 10 cells, respectively).

Renshaw cells may be activated synaptically by both cholinergic and non-cholinergic pathways, and in the present experiments these different synaptic inputs were differentiated by the use of the nicotinic antagonist dihydro- $\beta$ -erythroidine (DH $\beta$ E). D- $\alpha$ -Aminoadipate (40–100 nA for 4–20 min) had no effect on the DH $\beta$ E-sensitive excitation of 11/11 quiescent Renshaw cells evoked by ventral root stimulation. In contrast, DH $\beta$ E-resistant excitation evoked by dorsal root stimulation was markedly depressed by D- $\alpha$ -aminoadipate (20–90 nA for 3–5 min) on 5/5 of these cells (Fig. 2). The firing of a further 7 spontaneously active Renshaw cells was also depressed by either D- or DL- $\alpha$ -aminoadipate (10–80 nA).

Experiments on the isolated frog spinal cord have indicated that the whole of the depressant activity of DL- $\alpha$ -aminoadipate is due to the D isomer (our unpublished observations). In other experiments we have shown that D- $\alpha$ -aminoadipate has little or no effect on responses of the

isolated rat spinal cord to carbachol, substance P or nor-adrenaline and that the agent does not affect transmission in the rat superior cervical ganglion. These findings, together with the specificity shown by the agent in depressing chemically-induced and synaptically-evoked excitations in the present work, strongly suggest that D- $\alpha$ -amino adipate acts by a specific antagonism of amino acid-mediated synaptic excitation in the spinal cord. It follows that an acidic amino acid is probably released synaptically on to mouse dorsal horn interneurons and cat Renshaw cells in response to dorsal root stimulation. Since the actions of kainate and L-glutamate are less sensitive to the antagonist than those of NMDA and L-aspartate, our results support previous evidence<sup>4,8,9</sup> that the transmitter mediating such synaptic excitation may be L-aspartate.

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- Hall, J. G., McLennan, H. & Wheal, H. V. *J. Physiol., Lond.* **272**, 52–53P (1977).
- Biscoe, T. J. *et al. Eur. J. Pharmac.* **45**, 315–316 (1977).
- Johnston, G. A. R., Curtis, D. R., Davies, J. & McCulloch, R. M. *Nature* **248**, 804–805 (1974).
- McCulloch, R. M., Johnston, G. A. R., Game, C. J. A. & Curtis, D. R. *Expl Brain Res.* **21**, 515–518 (1974).
- Biscoe, T. J., Headley, P. M., Martin, M. R. & Sterling, C. A. *J. neurol. Sci.* **31**, 51–61 (1977).
- Davies, J. & Watkins, J. C. *Brain Res.* **130**, 364–368 (1977).
- Curtis, D. R. & Ryall, R. W. *Expl Brain Res.* **2**, 81–96 (1966).
- Davidoff, R. A., Graham, L. T., Shank, R. P., Werman, R. & Aprison, M. H. *J. Neurochem.* **14**, 1025–1031 (1967).
- Duggan, A. W. *Expl Brain Res.* **19**, 522–528 (1974).

## Axon conduction velocity modified by reinnervation of mammalian muscle

WHEN Buller *et al.*<sup>1</sup> observed that cross reinnervation of fast- and slow-twitch muscles could reverse their mechanical properties, the operations had been performed in the hope of finding changes in reflex connections. The changes in muscles were of great interest and have dominated this

field of research until recently, when Kuno *et al.* investigated some changes in motoneurone properties following axotomy and reinnervation. Retrograde changes of some electrical properties of the soma were observed which were reversed when the nerve re-established connections with the muscle. We report here some experiments on motor units in normal and reinnervated muscles, in which we have found that the conduction velocities of regenerated axons depend on the nature of the muscle which they reinnervate.

The initial operations were carried out on young adult cats of 1.5–2 kg body weight. Under anaesthesia, the nerves to flexor digitorum longus (FDL) and soleus muscles (fast- and slow-twitch respectively) were divided between fine ligatures. Each central end was reunited either to its own distal stump (self reinnervation) or to that of the other nerve (cross reinnervation).

For the final experiment either FDL or soleus was prepared for isometric recording and ventral roots were exposed by laminectomy. Functionally single axons were isolated by splitting the ventral roots with fine forceps as described elsewhere<sup>3,4</sup>. Only axons producing a response in the muscle under test were examined, but some of these would have branched to innervate the other muscle.

Axon conduction velocity was estimated from the latency of the antidromic action potential and the inter-electrode conduction distance (168–198 mm) measured *in situ* proximal to the neuroma. The stimulus was set at twice threshold for a 0.2-ms rectangular pulse. Axonal conduction velocity increases during regeneration but this could not affect the interpretation of our results since there were no significant differences between the post-operative survival periods of the self and cross re-innervated groups (Table 1b).

The axonal conduction velocities recorded in these experiments are displayed as the histograms of Fig. 1. In each panel, the open columns indicate axons which had originally innervated FDL muscles. In the control and self reinnervated groups these axons still innervated FDL muscle; in the cross reinnervated groups the 'FDL axons' innervated soleus muscle at the time of recording. In the same way, the hatched columns indicate soleus axons. A statistical analysis of the results is presented in Table 1a.

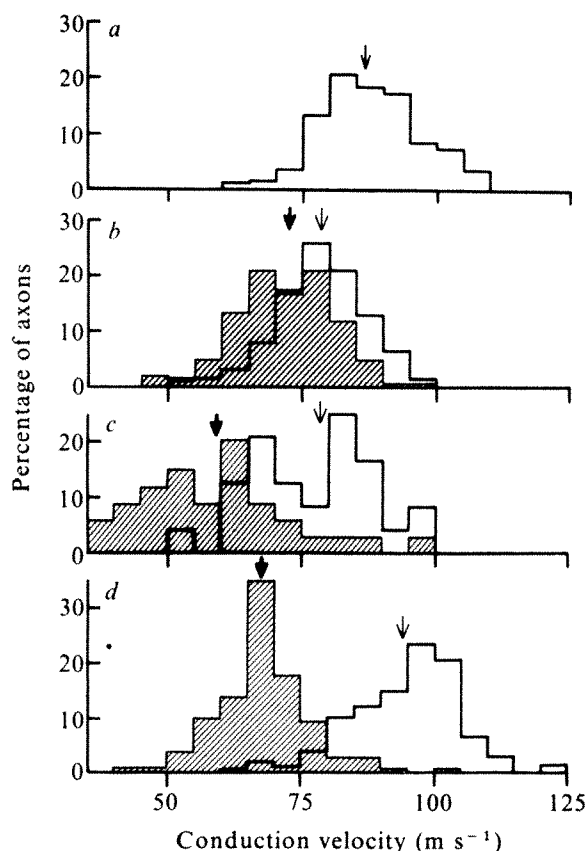
In normal muscles, FDL axons have a higher mean conduction velocity than those of soleus. After section and self reinnervation for 6 months, this difference persisted, although both groups of nerves conduct more slowly—at a mean velocity which was 83% of normal in FDL and 87% in soleus. This finding was anticipated, since slowing of conduction velocity proximal to the region of damage is well known.

In the FDL axons cross reinnervating soleus for 6 months, a reduction in conduction velocity to 83% of

Table 1 Statistical analyses of results of experimental reinnervation of muscle

Experimental group	Control	Self (6 month)	Cross (6 month)	Cross (2 yr)
<b>a, Conduction velocity (m s<sup>-1</sup>)</b>				
FDL	94.2 (205) ± 9.4	78.3 (24) * ± 10.1	78.5 (62) * ± 8.9	87.2 (115) *† ± 12.9
Soleus	67.5 (172) ± 8.0	58.7 (34) *† ± 14.1	71.9 (142) * ± 8.7	—
<b>b, Reinnervation period (d)</b>				
FDL	—	200 (5) ± 18	180 (5) ± 23	745 (5) ± 7
Soleus	—	215 (4) ± 20	198 (5) ± 15	—

The initial values are means; in parentheses are the number of axons (a) or of animals (b); ± s.d. Significant differences between means \* (comparison with corresponding control axons) and † (comparison with corresponding 6-month cross reinnervated axons, control group not indicated thus). In all these cases the differences by the *t*-test were significant at least at the 0.0001 level.



**Fig. 1** Distributions of conduction velocities of FDL (open columns, light arrows) and soleus (hatched columns, dark arrows) axons in control cats and after reinnervation operations. Arrows indicate group means. *a*, Cross reinnervation, 2 yr; *b*, cross reinnervation, 6 months; *c*, self reinnervation, 6 months; *d*, control.

control was observed, which was very close to that in the corresponding self reinnervated group. We have followed cross reinnervated FDL axons for 2 yr and found that although recovery progressed over this longer period it was still not complete.

In contrast the mean conduction velocity of soleus axons cross reinnervating FDL muscle at 6 months was greater by 7% than that of normal soleus axons. The mean conduction velocity of the cross reinnervated soleus axons was 24% greater than that of the self reinnervated axons; the difference was highly significant ( $t=6.9$ ,  $P<10^{-6}$ ).

The present results seem to differ from those of Kuno *et al.*<sup>2</sup>, who concluded that conduction velocities of soleus axons were restored to normal, both in self and in cross reinnervation, at 4–5 months. Their figures do, however, show more complete restoration following cross reinnervation and although this difference was not significant statistically (their numbers were smaller) it was in the same direction as ours and there may be no real discrepancy. Kuno *et al.*<sup>2</sup> did not examine cross reinnervated fast muscle nerves.

We conclude that the properties of reinnervating  $\alpha$ -motoneurons, as expressed in the conduction velocity of their axons above the neuroma, are influenced by the nature of the muscle into which they grow. In particular, axons which normally innervate a slow-twitch muscle may have their conduction velocity increased by being made to innervate a fast-twitch muscle. Presumably, the anatomical basis of this would be an increase in axon diameter. Two general hypotheses are possible. One involves a direct influence from the muscle as has been suggested by Kuno *et al.*<sup>2</sup>; alternatively, the effect might involve a change in afferent information from a muscle receiving a foreign innervation with a different pattern

of activity. The second type of hypothesis could be extended more easily to explain why the effects are asymmetrical between fast and slow muscle.

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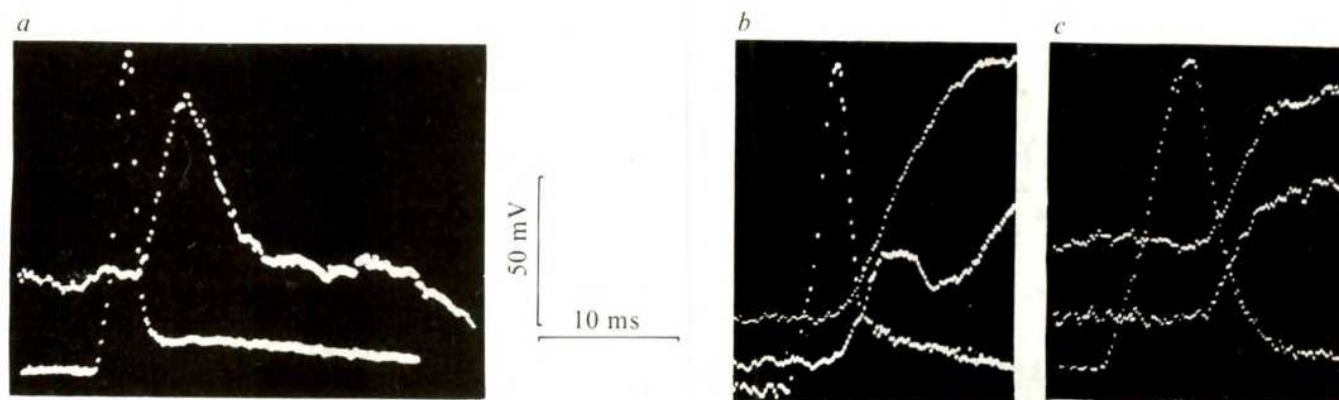
1. Buller, A. J., Eccles, J. C. & Eccles, R. M. *J. Physiol., Lond.* **150**, 417–439 (1960).
2. Kuno, M., Miyata, Y. & Munoz-Martinez, E. J. *J. Physiol., Lond.* **242**, 273–288 (1974).
3. Bagust, J., Knott, S., Lewis, D. M., Luck, J. C. & Westerman, R. A. *J. Physiol., Lond.* **231**, 87–104 (1973).
4. Bagust, J. & Lewis, D. M. *J. Physiol., Lond.* **237**, 91–102 (1974).

## Birefringence signals and calcium transients in skeletal muscle

TWITCHES in skeletal muscle are preceded and accompanied by changes in optical properties of the muscle fibres<sup>1–11</sup>. Measurements of birefringence give large signals which can be separated into two main components<sup>8–10</sup>: the first begins on the falling phase of the action potential, and reaches a peak at the onset of tension development; this is followed by a late signal which is thought to be associated with development of tension by the contractile proteins<sup>2,3,6</sup>. Since the early signal precedes tension development, it could provide an important tool for studying intervening steps in excitation-contraction coupling. This signal has recently been attributed to potential changes in the sarcoplasmic reticulum (SR) membrane, associated with  $\text{Ca}^{2+}$  release into the sarcoplasm<sup>6,10</sup>. We have simultaneously recorded birefringence signals and changes in intracellular  $\text{Ca}^{2+}$  concentration, using arsenazo III, in frog muscle fibres. The results show that the onset of the early birefringence signal coincides with the rise in sarcoplasmic  $\text{Ca}^{2+}$  concentration, and that both can be abolished by injecting EGTA to chelate the sarcoplasmic  $\text{Ca}^{2+}$ . This suggests that the early birefringence signal arises from some process dependent on the rise in sarcoplasmic  $\text{Ca}^{2+}$ , and is not caused by events in the SR associated with the calcium release mechanism.

Experiments were performed on the cutaneous pectoris muscle of *Rana temporaria*, using a bathing solution of the following composition (in  $\text{mmol l}^{-1}$ ): NaCl, 120; KCl, 2;  $\text{CaCl}_2$ , 2; phosphate buffer (pH 7.2), 3. Intracellular  $\text{Ca}^{2+}$  changes were measured with arsenazo III as previously described<sup>12,13</sup>. Briefly, the muscle was stretched sufficiently to block visible contraction, and a superficial fibre was impaled with two microelectrodes about 150  $\mu\text{m}$  apart, for voltage recording and dye injection. Arsenazo III was injected by iontophoresis. The dye pipette also served to initiate action potentials by passing 1-ms depolarising current pulses or, in some experiments, to voltage-clamp a region of the fibre. White light was focused on a spot of 80  $\mu\text{m}$  diameter between the pipettes, and transmitted through the fibre was diverted to photomultipliers. Calcium-dependent changes in fluorescence of arsenazo III were recorded by subtracting





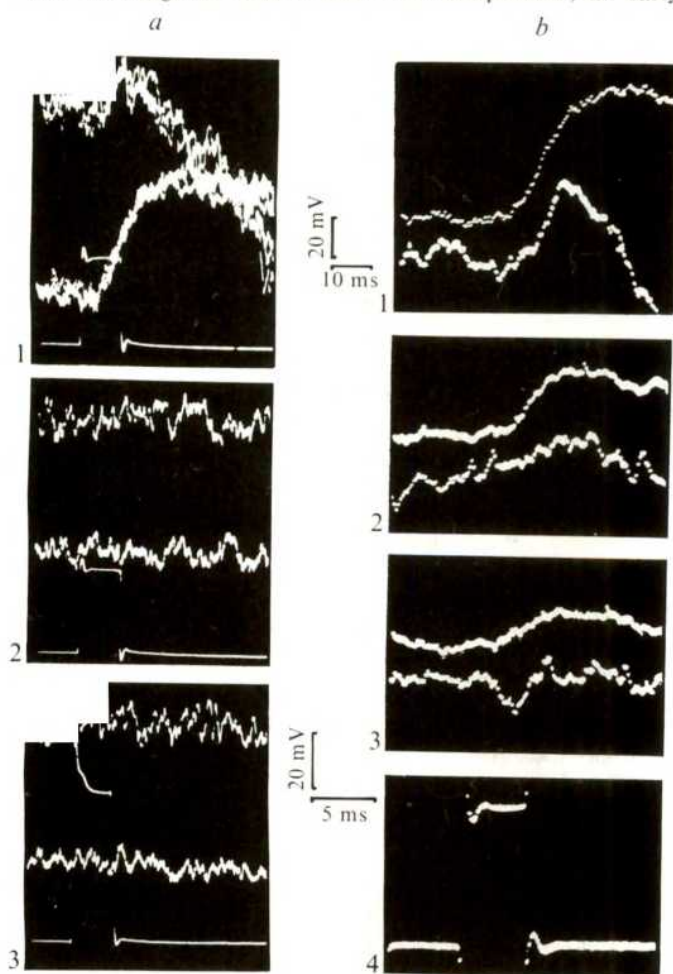
**Fig. 1** Changes in birefringence and light absorption of arsenazo III following action potentials. Traces are signal averages of 32, 16 and 24 sweeps in *a*, *b* and *c*, respectively. In all panels, the bottom trace is the membrane potential; birefringence changes are shown in the upper trace in *a* and in the middle traces in *b* and *c*. Changes in sarcoplasmic  $\text{Ca}^{2+}$  concentration detected with arsenazo III are shown in the top traces in *b* and *c*. Both optical records were low-pass filtered with a time constant of 1 ms, and are expressed as the change in light transmitted through the fibre, divided by the resting transmission. Fractional changes in light transmission;  $10^{-2}$  for the arsenazo III trace, and  $2.5 \times 10^{-3}$  for the birefringence. An upward deflection in both optical traces indicates a decrease in light intensity. Temperature: *a* and *b*, 10 °C; *c*, 4 °C.

the measurements of transmitted light at wavelengths of 532 and 602 nm, obtained by using two photomultipliers and interference filters. Birefringence changes were recorded with the third photomultiplier and two crossed polarisers, once placed in front of the light source and the other in front of the photomultiplier. The plane of polarisation was at 45° to the longitudinal axis of the muscle fibres<sup>1,8</sup>.

Preliminary experiments indicated that intracellular injection of arsenazo III did not affect the time course of the birefringence signals recorded following action potentials. The birefringence records show two components; an early

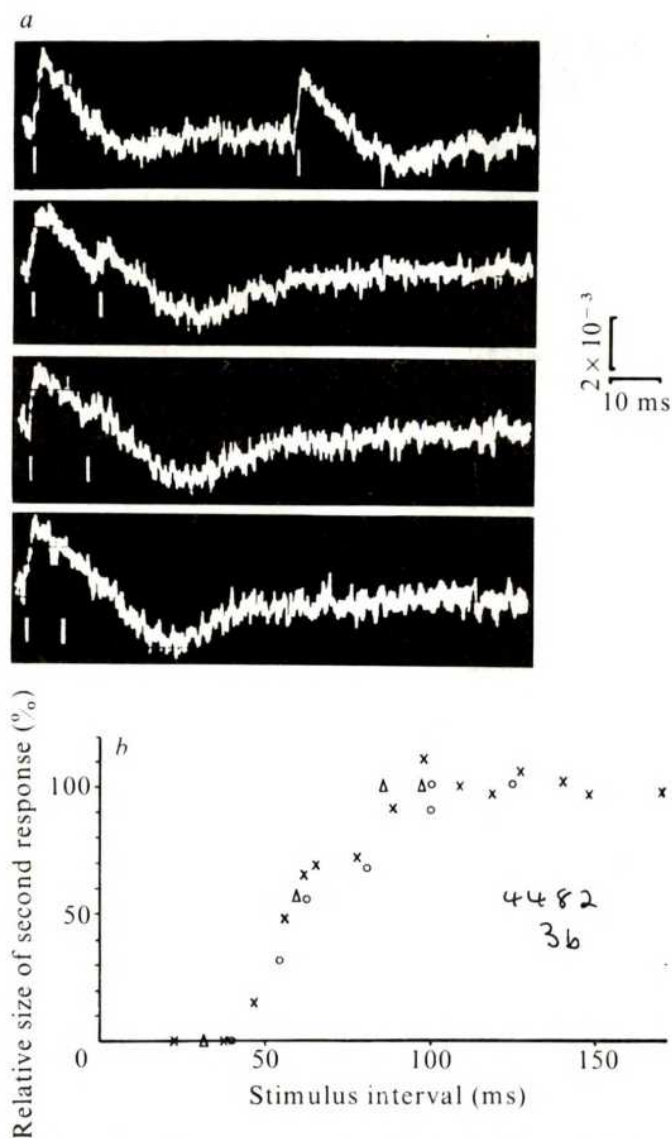
transient decrease in light intensity, followed by a signal with a similar time course to the movement artefact detected when one polariser was removed. This late component could appear as either an increase or a decrease in light intensity, depending critically on the positioning of the light spot. In a few fibres, the applied stretch sufficiently reduced the late signal to allow the early signal to be recorded with minimal distortion (Fig. 1*a*), but generally the early signal was obscured after its peak (Fig. 1*b* and *c*). At 10 °C the early signal takes off within 2 ms of the foot of the action potential, and reaches a peak within 6 ms. The onset of the birefringence signal coincides with the onset of the calcium response as detected by the arsenazo III, and the latencies of both are affected to the same extent by changes in temperature (Fig. 1*b* and *c*).

Intracellular injection of EGTA provides a means of exploring the relationship between the events underlying these optical changes, since it reduces the increase in sarcoplasmic  $\text{Ca}^{2+}$  by binding the  $\text{Ca}^{2+}$  ions released by the SR (ref. 14). A micropipette containing 0.2 M EGTA (buffered to pH 7 with KOH) was inserted with its tip in the centre of the measuring light spot. A depolarising backing current (10 nA) was applied to the pipette while the control records were taken. To minimise movement artefacts resulting from contraction of distant regions of the fibre, action potentials were blocked with tetrodotoxin ( $10^{-6}$  g ml<sup>-1</sup>); tetraethylammonium bromide (30 mM) was added to the medium and the membrane was voltage-clamped in the recording area. The fibre was stimulated by giving 5- or 10-ms depolarising pulses and EGTA was injected by



**Fig. 2** Effect of EGTA on the birefringence signals and  $\text{Ca}^{2+}$  transients. The fibres were stimulated with 10-ms depolarising pulses in *a* and 5-ms pulses in *b*; tetrodotoxin ( $10^{-6}$  g ml<sup>-1</sup>) and tetraethylammonium (30 mM) were present in the medium. *a* and *b* are records from two different fibres. *a*, Bottom trace is membrane potential, middle trace is arsenazo III responses and top trace shows birefringence changes. *a*1 shows control responses (three superposed sweeps); *a*2 and *a*3 show the blockade of the optical signals following injection of EGTA for 8 min with an average current of 50 nA. Resting potential: 85 mV. *b*, Changes in dye absorption (upper trace in *b*1–*b*3) and in birefringence (lower trace in *b*1–*b*3) induced by depolarising pulses (shown in *b*4). Records are averages of 48 sweeps. EGTA was applied for 2 min at 40 nA between *b*1 and *b*2, and for 2 min at 60 nA between *b*2 and *b*3. Upper calibration bars apply to fibre *a*: horizontal, 10 ms; vertical, 20 mV,  $2 \times 10^{-3}$  (arsenazo III response) and  $4 \times 10^{-3}$  (birefringence changes). Lower calibration bars refer to fibre *b*: horizontal, 5 ms; vertical, 20 mV,  $4 \times 10^{-3}$  (arsenazo III) and  $2 \times 10^{-3}$  (birefringence). Temperature: *a*, 7 °C; *b*, 6 °C.





**Fig. 3** Birefringence response to action potentials. *a*, Single sweeps showing four runs at stimulus intervals of 250, 65, 55 and 35 ms; vertical bars under the birefringence traces indicate the peak time of the action potentials; temperature, 4 °C. *b*, Relative size of the birefringence signal elicited by the second pulse as a function of stimulus interval. Data from three different fibres; temperature, 4 °C.

applying a small hyperpolarising current through the micro-electrode. The results are shown in Fig. 2. Blockade of the rise in free sarcoplasmic  $\text{Ca}^{2+}$  was accompanied by inhibition of both the early and the late birefringence signals (Fig. 2*a*); the intensity of the block could be graded by progressive increase in the amount of EGTA injected (Fig. 2*b*). In one fibre, a partial recovery of the birefringence and calcium signals was observed when recording was continued for about 30 min after injection. The most direct interpretation of these results is that the birefringence signals were abolished as a consequence of the reduction in size of the  $\text{Ca}^{2+}$  transient due to  $\text{Ca}^{2+}$  binding to the EGTA (ref. 14), but the possibility cannot be entirely ruled out that the EGTA additionally interfered in some way with the  $\text{Ca}^{2+}$  release mechanism.

Experiments with paired stimuli provide further evidence against the early birefringence signal being associated with the calcium release mechanism. When the interval between the two stimuli was less than 40 ms (at 4 °C), no early birefringence change was detected following the second stimulus (Fig. 3*a*). The curve relating the size of the second response to the stimulus interval (Fig. 3*b*) shows a steep slope between 50 and 90 ms, with recovery to 50% of the

control value in about 60 ms. In contrast,  $\text{Ca}^{2+}$  transients measured with arsenazo III summate almost linearly with stimulus intervals as close as 20 ms (R. Miledi, I.P. and G. Schalow, unpublished data).

The abolition of the birefringence signals by intracellular injection of EGTA strongly suggests that the early signal is not associated with the  $\text{Ca}^{2+}$  release process by the SR, but is instead secondary to the increase in free sarcoplasmic  $\text{Ca}^{2+}$  concentration. This view is supported by the coincidence in time courses of the  $\text{Ca}^{2+}$  transient and the early birefringence signal, and by the experiment with the paired stimuli. The simplest interpretation of the paired stimulus experiment is that the early birefringence signal arises from a process which is fully saturated by the rise in sarcoplasmic  $\text{Ca}^{2+}$  concentration resulting from a single action potential. A possible mechanism for this early birefringence signal is a conformational change in the thin filament caused by the  $\text{Ca}^{2+}$  binding to troponin<sup>3,10</sup>. Latency relaxation and elongation have recently been attributed to a lengthening of the thin filament resulting from  $\text{Ca}^{2+}$  binding, and the time course of these phenomena parallels that of the early optical changes<sup>1,3</sup>.

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- Hill, D. K. *J. Physiol., Lond.* **108**, 292–302 (1949).
- Eberstein, A. & Rosenfalck, A. *Acta physiol. scand.* **57**, 144–166 (1963).
- Barry, W. H. & Carnay, L. D. *Am. J. Physiol.* **217**, 1425–1430 (1969).
- Carnay, L. D. & Barry, W. H. *Science* **165**, 608–609 (1969).
- Bezanilla, F. & Horowicz, P. *J. Physiol., Lond.* **246**, 709–735 (1975).
- Baylor, S. M. & Oetliker, H. *Nature* **253**, 97–101 (1975).
- Oetliker, H., Baylor, S. M. & Chandler, W. K. *Nature* **257**, 693–696 (1975).
- Baylor, S. M. & Oetliker, H. *J. Physiol., Lond.* **264**, 141–162 (1977).
- Baylor, S. M. & Oetliker, H. *J. Physiol., Lond.* **264**, 163–198 (1977).
- Baylor, S. M. & Oetliker, H. *J. Physiol., Lond.* **264**, 199–213 (1977).
- Kovács, L. & Schneider, M. F. *Nature* **265**, 556–560 (1977).
- Miledi, R., Parker, I. & Schalow, G. *Proc. R. Soc. Lond. B* **198**, 201–210 (1977).
- Miledi, R., Parker, I. & Schalow, G. *J. Physiol., Lond.* **269**, 11–13P (1977).
- Ashley, C. C. *Am. Zool.* **7**, 647–659 (1967).

## Calcium requirement for axoplasmic transport in mammalian nerve

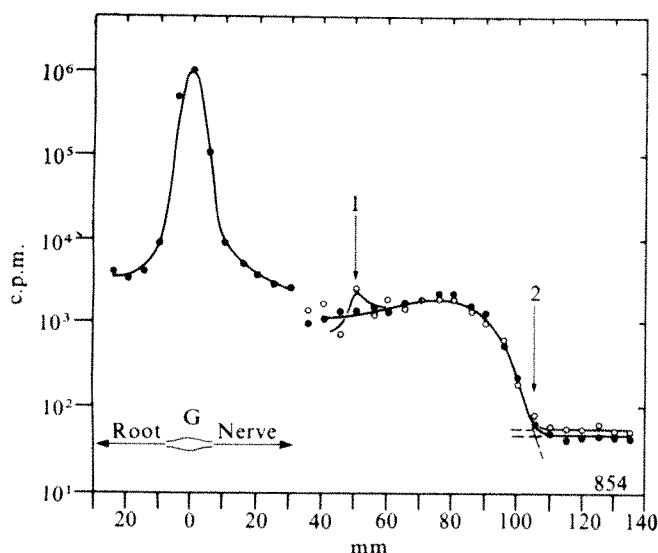
To account for fast axoplasmic transport, the movement of materials in nerve fibres, a model has been advanced in analogy to the sliding filament mechanism of muscle contraction<sup>1</sup>. We have shown in *in vitro* studies that transport is closely dependent on oxidative metabolism and a continual supply of ATP which could be hydrolysed by the  $\text{Mg}^{2+}$ - $\text{Ca}^{2+}$  ATPase present in nerve<sup>2</sup>. One might, therefore, expect a dependence of transport on either  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ . But, in previous studies of axoplasmic transport *in vitro*, we and others found transport to continue as usual when nerves were placed in incubation media free of divalent cations<sup>3–5</sup>. Some support for the involvement of  $\text{Ca}^{2+}$  in transport was provided by the fact that a block of transport was found with 50 mM oxalate, presumably by a binding of intracellular  $\text{Ca}^{2+}$  (ref. 1) and the block of organelle movement observed in single fibres exposed to 10 mM EDTA<sup>6</sup>. We report here that clear evidence for a participation of  $\text{Ca}^{2+}$  in axoplasmic transport was revealed when a desheathed nerve preparation was used.

The perineurial sheath acts as an effective permeability barrier in nerve<sup>7,8</sup>, retaining ions including  $\text{Ca}^{2+}$  within the

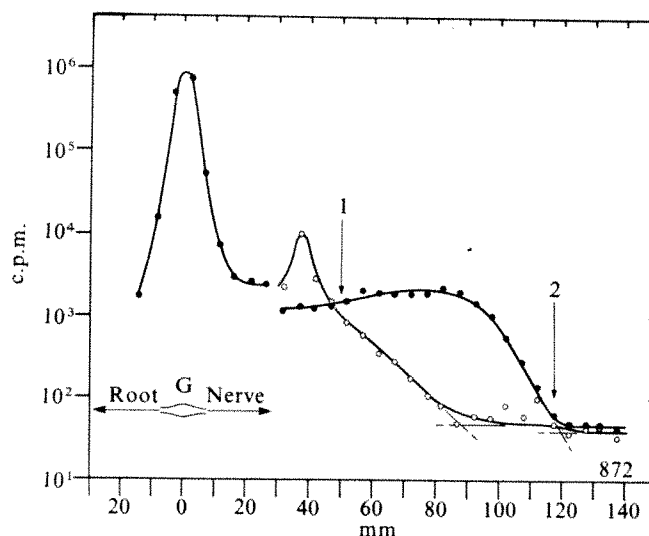
endoneurial space. This could account for the apparent insensitivity of transport *in vitro* to a deficiency of  $\text{Ca}^{2+}$  in the medium when intact nerves are used. To examine the effect of  $\text{Ca}^{2+}$  on transport *in vitro*, it was essential that a long length of nerve be desheathed so as to allow the effects of a given concentration of  $\text{Ca}^{2+}$  or its absence from the medium to be assessed over incubation periods lasting at least 4 h. The desheathed nerve preparation used was the peroneal branch of the cat sciatic nerve from which a sufficiently long length of perineurial sheath can be readily removed.

In accord with our usual procedure, the L7 dorsal root ganglia were injected with  $20 \mu\text{Ci}$  of  $^3\text{H}$ -leucine which is rapidly taken up by the cell bodies and incorporated into proteins and polypeptides<sup>9</sup>. Two hours were allowed for proteins and polypeptides containing incorporated  $^3\text{H}$ -leucine to move down the fibres of the tibial and peroneal nerve branches. The sciatic nerves were removed and the peroneal branches desheathed over a distance from 35 mm to 130 mm from the L7 dorsal root ganglia. The sciatic nerves with their desheathed peroneal and sheathed tibial branches attached were then put into flasks containing 20 ml of the desired solution and incubated for 3–5 h at  $38^\circ\text{C}$  while vigorously oxygenated with 95%  $\text{O}_2$  + 5%  $\text{CO}_2$ . The outflow patterns in both the peroneal and tibial branches were then separately determined by cutting each in equal 5 mm portions and assessing their content of labelled activity<sup>10</sup>.

With Ringer solution as the *in vitro* medium, or with isotonic NaCl or isotonic sucrose solution to which 5 mM  $\text{Ca}^{2+}$  was added, the outflow in both the desheathed peroneal and tibial nerves was similar (Fig. 1). The rate of transport



**Fig. 1** Transport in medium containing 5 mM  $\text{CaCl}_2$ . Outflow in the desheathed peroneal branch (○) and the sheathed tibial branch (●) is shown. After 2 h of downflow *in vivo*, the peroneal nerve was desheathed and the nerve placed in an *in vitro* medium of 5 mM  $\text{CaCl}_2$  and 140 mM NaCl for an additional 4 h. The points represent labelled materials present in 5 mm segments cut from the dorsal root, L7 ganglion (G) and nerve. Distal to 30 mm from the ganglion, each branch is cut individually and thus outflow in the tibial (T) and peroneal (P) branches can be shown separately. Arrow 1 indicates the upper end of the desheathed region and arrow 2, the front of the crests of transported material expected in both nerves. The crests seem to have the same height in this example. It should be noted that the logarithmic scale serves to diminish amplitude differences in the two branches. In general, a greater amount of labelled activity was usually found transported in the tibial branch since it is the larger of the two. A small degree of damming of activity is seen above the upper site where desheathing was initiated (arrow 1). Activity in counts per minute (c.p.m.) is given on the ordinate on a logarithmic scale. The position of sections is shown in mm from zero taken at the peak of activity in the ganglion.



**Fig. 2** Transport in Ca-free medium. Outflow in a sciatic nerve *in vitro* and its desheathed peroneal branch (○) and sheathed tibial branch (●) is shown following injection of the L7 ganglion with  $^3\text{H}$ -leucine. Downflow for 2 h was allowed in the animal before the sciatic nerve was removed, the peroneal branch desheathed and the nerve placed in an *in vitro* medium containing Ca-free isotonic NaCl. Above arrow 1, approximately where the peroneal nerve is desheathed, a peak of dammed activity is seen. This is followed by a steep descent to baseline at about 85 mm. Conversely, the tibial nerve shows a typical axoplasmic transport outflow. Arrow 2 marks the expected distance.

in both sheathed and desheathed branches determined in 25 experiments was close to  $410 \text{ mm d}^{-1}$ , the rate previously found to be characteristic of fast axoplasmic transport<sup>1</sup>. A somewhat more sloping crest was seen at the advancing fronts in the desheathed nerves at lower  $\text{Ca}^{2+}$  concentrations of 1.5 to 3 mM. The addition of 4 mM  $\text{K}^+$  to the medium with 1.5  $\text{Ca}^{2+}$  gives a downflow closer to the usual pattern, indicating some participation of  $\text{K}^+$  in maintaining transport at these  $\text{Ca}^{2+}$  concentrations.

With desheathed nerves placed in a Ca-free media, axoplasmic transport was blocked in the desheathed peroneal nerve (Fig. 2). The outflow of activity shows some damming at the upper end of the desheathed region followed by a gradual drop in the front of advancing activity until it meets the baseline level at which time a complete block of transport ensues. Subtracting the time of 2 h during which downflow took place in the animal, the block within the desheathed portion of the nerve was found to develop in 2.6 h of exposure to the Ca-free media (a, Table 1). A similar block was found when 4 mM EGTA was added to a Ca-free solution (b, Table 1) and to a Ringer solution in line with  $\text{Ca}^{2+}$  being the key factor. Little effect of variation of buffer and over pH 6.0–7.5 was found.

Transport was not maintained with  $\text{Mg}^{2+}$  in the incubation medium instead of  $\text{Ca}^{2+}$  (c, Table 1), indicating that  $\text{Ca}^{2+}$  is specifically required for the maintenance of axoplasmic transport. But, there was a small but significant prolongation of the time to block with  $\text{Mg}^{2+}$  present over the Ca-free medium which could indicate a partial effect of  $\text{Mg}^{2+}$ .

While axoplasmic transport was blocked in the desheathed nerve in the Ca-free medium, a normal appearing outflow was present in the sheathed tibial branch (Fig. 2). This is consistent with a large number of our previous observations showing an apparent lack of effect of  $\text{Ca}^{2+}$  depletion on axoplasmic transport in sheathed sciatic nerves *in vitro* and supports the view that  $\text{Ca}^{2+}$  is retained within the intact perineurial sheath.

We consider that the removal of  $\text{Ca}^{2+}$  from the medium lowers the level of free  $\text{Ca}^{2+}$  normally present in the fibres of desheathed nerves below that required for transport. The



level of free  $\text{Ca}^{2+}$  in the axoplasm of mammalian nerve fibres is not, however, known. If it is similar to that present in other cells, particularly the axoplasm of cephalopod giant axons, we would expect its concentration to be approximately  $10^{-7}$  M (refs 11, 12). This low level of  $\text{Ca}^{2+}$  is considered to be maintained by several mechanisms in the face of a constant influx of  $\text{Ca}^{2+}$ . These include a  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange carrier in the membrane<sup>13</sup> which may possibly be dependent on ATP (refs 14, 15), a sequestration of  $\text{Ca}^{2+}$  within the mitochondrion<sup>16,17</sup> and likely as well in the endoplasmic reticulum<sup>18</sup>. To this list we may add the possibility of a Ca-binding protein (CaBP) recently found in cat sciatic nerve<sup>19</sup>.

An involvement of mitochondria in the block of transport found in nerves placed in Ca-free media was indicated by a reduction in ATP and creatine phosphate levels. In nine nerves, the combined levels of ATP and creatine phosphate ( $\sim\text{P}$ ) showed a decrease of approximately 22%, from control levels of  $1.10 \pm 0.30 \mu\text{M}$  per g weight in a Ca-medium to  $0.86 \pm 0.18 \mu\text{mol g}^{-1}$  in a Ca-free media which took place after 3 h. This degree of reduced  $\sim\text{P}$  is not, however, likely in itself to account for the block of axoplasmic transport. In six desheathed nerves exposed to a Ca-free medium, we saw action potentials still remaining 2 h after a block of axoplasmic transport. This shows that the  $\sim\text{P}$  remaining was sufficient to supply the Na-pump required to maintain excitability and presumably there should be an adequate supply for the transport mechanism as well. A block of axoplasmic transport and action potentials was found to occur only when  $\sim\text{P}$  levels were reduced by 50% (ref. 20).

**Table 1** Block time of axoplasmic transport on desheathed nerves in  $\text{Ca}^{2+}$ -free media

	Condition	n	Block time (h)
a	$\text{Ca}^{2+}$ -free	24	$2.6 \pm 0.79$
b	EGTA + $\text{Ca}^{2+}$ -free	11	$3.0 \pm 0.36$
c	5 mM $\text{Mg}^{2+}$	15	$3.5 \pm 0.48$

The time to block is expressed in hours calculated from the following equation:  $(D_2 - D_1) \text{ mm} / 17.4 \text{ mm h}^{-1}$ , where  $D_2$  = total distance the labelled materials moved down in the sheathed and desheathed part of the peroneal branch;  $D_1$  = distance moved in the sheathed part of the peroneal branch. All media containing NaCl or sucrose so that the isotonicity was adjusted to approximately 300 mOsm. 10 mM  $\text{NaHCO}_3$  was added as buffer (pH 6.7). n, No. of experiments; P = significance. The significant levels were analysed by t-test. P values for groups a, c < 0.001, for b, c < 0.01, and for a, b > 0.2. Values are mean  $\pm$  s.d.

Transport in the desheathed nerve also showed a progressive failure of transport with higher than normal levels of  $\text{Ca}^{2+}$  present in the incubation medium. This was seen to occur in the range of 25–100 mM  $\text{Ca}^{2+}$ . At the highest levels of  $\text{Ca}^{2+}$  (95–100 mM) transport was blocked in a little less than 2 h of incubation. The actual amount of  $\text{Ca}^{2+}$  entering the fibres and the resulting concentration change in the axoplasm is unknown. These findings suggest, however, that  $\text{Ca}^{2+}$  must be regulated within some limited range for normal transport.

An excess of  $\text{Ca}^{2+}$  entering the fibres could cause a block of transport by interfering with ATP production<sup>16,17</sup> or its utilisation by the  $\text{Mg}^{2+}$ - $\text{Ca}^{2+}$  ATPase. In addition, at the higher levels of  $\text{Ca}^{2+}$ , preliminary electron microscopic studies have shown a marked depletion of microtubules in the fibres<sup>20</sup>, which may be related to the disassembly of isolated microtubules by  $\text{Ca}^{2+}$  (S.O., S.-Y.C., R. Jersild and V. McAdoo, unpublished and ref. 21). On the basis of the transport filament hypothesis a block of ATP production or its utilisation, and at higher  $\text{Ca}^{2+}$  levels the disassembly of microtubules, would all be possible causes for the block of axoplasmic transport observed.

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- Ochs, S. *Science* **176**, 252–260 (1972).
- Khan, M. A. & Ochs, S. *Brain Res.* **81**, 413–426 (1974).
- Banks, P., Mayor, D. & Mraz, P. *J. Physiol., Lond.* **229**, 383–394 (1973).
- Ochs, S. & Smith, C. *J. Neurobiol.* **6**, 85–102 (1975).
- Hammerschlag, R., Dravid, A. R. & Chiu, A. Y. *Science* **188**, 273–275 (1975).
- Kirkpatrick, J. B. & Rose, R. E. *Trans. Soc. Neurosci.* **2**, 255 (1972).
- Crescitelli, F. *Am. J. Physiol.* **166**, 229–240 (1951).
- Krujević, K. *J. Physiol., Lond.* **128**, 473–488 (1955).
- Ochs, S., Sabri, M. I. & Johnson, J. *Science* **163**, 686–687 (1969).
- Ochs, S. *J. Physiol., Lond.* **227**, 627–645 (1972).
- Baker, P. F. *Prog. Biophys. molec. Biol.* **24**, 177–223 (1972).
- Blaustein, M. P. *Rev. Physiol. Biochem. Pharm.* **70**, 33–82 (1974).
- Brinley, F. Jr, Spangler, S. G. & Mullins, L. J. *J. gen. Physiol.* **66**, 223–250 (1975).
- Baker, P. F. *Fedn Proc.* **35**, 2589–2595 (1976).
- DiPolo, R. *Fedn Proc.* **35**, 2579–2582 (1976).
- Lehninger, A. L. *Biochemistry*, 2nd edn (Worth, New York, 1975).
- Carafoli, E. & Crompton, M. *Symp. Soc. exp. Biol.* **30**, 89–115 (1976).
- Stockel, M. E., Hindelang-Gertner, C., Dellman, H. D., Porte, A. & Stutinsky, F. *Cell Tiss. Res.* **157**, 307–322 (1975).
- Iqbal, Z. & Ochs, S. *Soc. Neurosci. Abst.* **2**, 47 (1976).
- Ochs, S. *Fedn Proc.* **33**, 1049–1058 (1974).
- Weisenberg, R. C. *Science* **177**, 1104–1105 (1972).
- Shelanski, M. L., Gaskin, F. & Cantor, C. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 765–768 (1973).

## Ionic radius selectivity of skeletal muscle membranes

THERE have been several reports<sup>1–3</sup> that the trivalent lanthanide cations bind to sites on membranes that are normally occupied by  $\text{Ca}^{2+}$ . In skeletal muscle,  $\text{Ca}^{2+}$  is important for the functions of both the outer sarcolemma<sup>4</sup> and the internal sarcoplasmic reticulum<sup>5,6</sup>. Here I report the inhibitory effects of 13 lanthanide ions on these  $\text{Ca}^{2+}$ -mediated functions. The relationship between ionic radius and the degree of inhibition is different in these two membranes. There are features of the data which are difficult to explain by assuming that charge density alone determines cation recognition at the membrane surface. But it would also be too simplistic to suggest that the lanthanide ions are selected only on the basis of ionic radius.

One of the main functions of the sarcolemma is the propagation of the action potential over the surface of the muscle fibre, causing it to contract. It has been reported that the maintenance of the action potential depends on membrane-bound  $\text{Ca}^{2+}$  and that this  $\text{Ca}^{2+}$  is specifically displaced by lanthanum ions<sup>4</sup>. Lanthanide ions have been shown<sup>7</sup> to exert a strong inhibition on the twitch response of small bundles (3–5 fibres) of toad semitendinosus fibres. The site of action of these ions seems to be the sarcolemma because they have been localised there and because removal of the sarcolemma by glycerination substantially raises the threshold concentration needed to inhibit contraction<sup>7</sup>.

Inhibition of the twitch response is most conveniently expressed as the concentration of lanthanide ion required to produce 50% inhibition of the twitch tension. Figure 1 shows that most of the lanthanide ions have about the same effect on twitch tension except for three,  $\text{Tm}^{3+}$ ,  $\text{Er}^{3+}$  and to a lesser extent  $\text{Ho}^{3+}$ , which are required in significantly higher concentrations in order to effect a 50% reduction in the contractile response. These ions have ionic radii of 99, 100 and 102 pm respectively and therefore correspond exactly with the published<sup>8</sup> radius of  $\text{Ca}^{2+}$  (100 pm). These data could be interpreted to mean that the skeletal muscle sarcolemma possesses an extremely acute ability to detect,

within a few pm, the ionic radii of multivalent cations such as the trivalent lanthanide ions.

The acute sensitivity of the sarcolemma to ionic radii of the lanthanide ions raises the question of whether this property is shared with other muscle membranes. Skeletal muscle sarcoplasmic reticulum is primarily concerned with the release of  $\text{Ca}^{2+}$  during activation of the fibre and with the subsequent recovery of  $\text{Ca}^{2+}$  associated with relaxation. *In vivo* it exists as a complex of flat fenestrated sheets and sacs surrounding the myofibrils. It can, however, be isolated in the form of vesicles which have been well characterised<sup>5</sup>. The membranes of these vesicles are structurally specialised and can transport  $\text{Ca}^{2+}$  against concentration gradients of up to 10,000 by the hydrolysis of Mg-ATP (ref. 6). Recent results<sup>3</sup> have shown that (1) lanthanide ions such as  $\text{Gd}^{3+}$  cannot penetrate the vesicles provided that their membranes are not damaged; (2)  $\text{Gd}^{3+}$  inhibits the  $\text{Ca}^{2+}$ -stimulated  $\text{Mg}^{2+}$ -dependent ATPase activity associated with  $\text{Ca}^{2+}$

uptake; and (3) the concentration dependence of this inhibition is less sharp than that observed in the sarcolemma experiments<sup>7</sup>. Therefore a series of experiments analogous to those described with the sarcolemma was undertaken. The data (Fig. 1) are a contrast to the sarcolemma experiments. There is no abrupt effect near the radius of  $\text{Ca}^{2+}$ . Instead, there is a roughly linear relationship between ionic radius and inhibition of sarcoplasmic reticulum Mg-ATPase activity.

Now that there are data on the ionic radius dependence of lanthanide ion inhibition of the sarcoplasmic reticulum, they can be compared with those from the sarcolemma experiments<sup>7</sup>. Clearly the two membranes behave quite differently in the presence of the lanthanide ions. The sarcolemma exhibits a sharp break at the ionic radius of  $\text{Ca}^{2+}$  which is absent from the sarcoplasmic reticulum data. The ionic milieu inside the muscle fibre is essentially constant, having high concentrations of  $\text{Mg}^{2+}$  and  $\text{K}^{+}$  and low  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$ . Only during activation is it necessary for the outer surface of the sarcoplasmic reticulum membrane to distinguish between  $\text{Ca}^{2+}$  (100 pm) and  $\text{Mg}^{2+}$  (49 pm). The outer surface of the sarcolemma is, however, confronted with high concentrations of  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  and low  $\text{K}^{+}$ , and there may be other cations present in the fluid around the muscle cells which will complicate the need for ionic selectivity. Perhaps it is not surprising that the sarcolemma has a more acute ability to recognise cations the size of  $\text{Ca}^{2+}$  and that the sarcoplasmic reticulum exhibits only a broad selectivity with respect to ionic radius.

What information do these data yield about the mechanism by which sites on muscle membranes recognise cations like  $\text{Ca}^{2+}$ ? The lanthanide ions in some respects resemble  $\text{Ca}^{2+}$ , for not only do their ionic radii overlap, but both exhibit purely ionic interactions under biological conditions<sup>11</sup>. It is tempting to conclude that these membranes select multivalent cations on the basis of ionic radius as the data (particularly the sarcolemma experiments) suggest. But all the lanthanide ions inhibit the  $\text{Ca}^{2+}$ -mediated functions in these membranes and so it seems that these membranes can distinguish between  $\text{Ca}^{2+}$  and those lanthanide ions with nearly similar ionic radii. Moreover, although the term "ionic radius" is valuable as a generalised property of ions, it is perhaps misleading if interpreted too precisely, for in reality there is no radius, but rather a set of dimensions which can vary depending on the ligand<sup>8,9</sup>. On the other hand, charge density<sup>10</sup> also seems unlikely to be the criterion for selecting ions like  $\text{Ca}^{2+}$ , since the larger lanthanide ions have approximately the same charge density as  $\text{Ca}^{2+}$  but are not apparently distinctive in their effects on the sarcolemma membrane. If neither ionic radius nor charge density alone can account for these data then perhaps some other explanation should be sought.

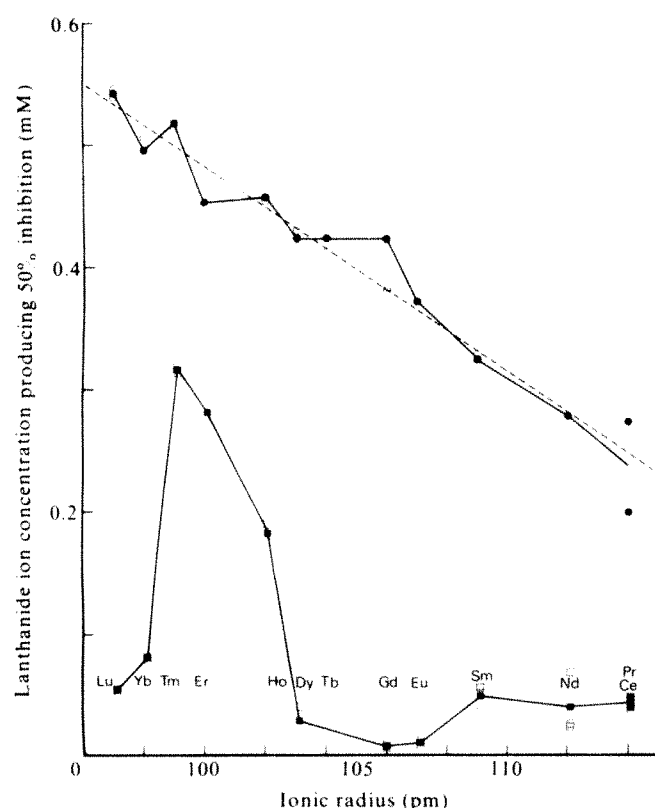
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1. Mela, L. *Biochemistry* **8**, 2481-2486 (1969).
2. Lehninger, A. L. & Carafoli, E. *Archs Biochem. Biophys.* **143**, 506-515 (1971).
3. dos Remedios, C. G. *J. Biochem.* **81**, 703-709 (1977).
4. Andersson, K. E. & Edman, K. A. P. *Acta physiol. scand.* **90**, 113-123 (1974).
5. Inesi, G. *Rev. Biophys. Bioengng* **1**, 191-210 (1972).
6. Hasselbach, W. *Biophys. Struct. Mech.* **3**, 43-54 (1977).
7. Hambly, B. D. & dos Remedios, C. G. *Experientia* **33**, 1042-1044 (1977).
8. Shannon, R. D. & Prewitt, C. T. *Acta Crystallogr.* **B25**, 925-946 (1969).
9. Williams, R. J. P. *Symp. Soc. exp. Biol.* **30**, 1-17 (1976).
10. Diamond, J. M. & Wright, E. M. *A. Rev. Physiol.* **31**, 581-646 (1969).
11. Barry, C. D., North, A. C. T., Glasel, J. A., Williams, R. J. P. & Xavier, A. V. *Nature* **232**, 236-245 (1971).



**Fig. 1** Relationship between ionic radius of the lanthanide ions (assuming a coordination number of 8 (ref. 11)) and the functions of two different membrane systems from vertebrate skeletal muscle. The upper curve represents the inhibition of  $\text{Ca}^{2+}$ -stimulated Mg-ATPase activity of isolated rabbit sarcoplasmic reticulum vesicles, expressed as the concentration of added lanthanide ion required to inhibit this activity by 50%. This concentration dependence ( $0.5 \times 10^{-4}$ – $5.0 \times 10^{-4}$  M lanthanide ion) was determined at least twice for each of the 13 lanthanide ions ( $\circ$ ) and the solid line joins their means ( $\bullet$ ). The dotted line is the linear regression ( $r=0.972$ ) fitted to the means. Conditions for these experiments were: KCl, 0.1 M; HEPES, 0.01 M; ATP, 2 mM;  $\text{MgCl}_2$ , 2.5 mM; sarcoplasmic reticulum protein, 0.1 mg ml<sup>-1</sup>; pH 7.0 at 25 °C (see ref. 3 for further details). The lower curve relates the ionic radii of the 12 lanthanide ions tested to the added concentration of each ion required to inhibit twitch tension by 50%. This concentration dependence ( $0.2 \times 10^{-4}$ – $10 \times 10^{-4}$  M lanthanide ion) was determined at least twice for each of the ions ( $\square$ ) and their means ( $\blacksquare$ ) have been joined by the solid line. Experimental conditions were: 3–5 toad semitendinosus fibres supramaximally stimulated in NaCl, 115 mM; KCl, 2.5 mM;  $\text{CaCl}_2$ , 1.8 mM; HEPES, 5 mM; pH 7.0 at 25 °C (for further details see ref. 7). The binding of the lanthanide ions to either the sarcolemma or the sarcoplasmic reticulum was found to be completely reversible.

## Control of interaction of spectrin and actin by phosphorylation

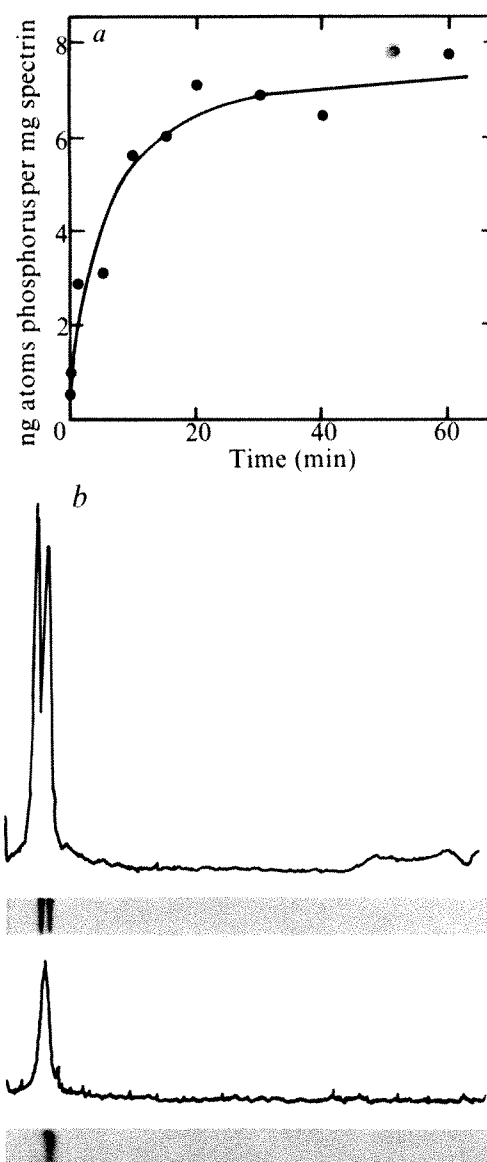
SPECTRIN is a high molecular weight protein located on the cytoplasmic surface of the mammalian erythrocyte membrane, from which it may be readily liberated by extraction with solutions of low ionic strength. It is thought to be a major structural element of the cell and to play a critical part in maintaining its discoid shape and characteristic viscoelastic properties. Birchmeier and Singer<sup>1</sup> have shown that changes in the shape of red cell ghosts are associated with the phosphorylation of a single serine residue on one of the two spectrin subunits, and have suggested that this might provide a basis for the well-known control of red cell shape by ATP<sup>2</sup>. The erythrocyte membrane also contains actin, which is present in approximately equimolar proportions to the spectrin dimer (molecular weight 500,000 (ref. 3)). We have shown previously<sup>4</sup> that there is a specific interaction between these two proteins, which reveals itself in the ability of spectrin to provoke the polymerisation of muscle actin *in vitro*. We show here that this effect depends on the phosphorylation of spectrin. Furthermore, in an undispersed mixture of spectrin and its cognate actin such phosphorylation causes the formation of a gel. The striking parallel between these results and the previously demonstrated effects of phosphorylation *in situ* suggests that the shape of the cell is controlled primarily by the actin-spectrin complex.

At least two kinase activities have been recognised in human erythrocyte membranes<sup>5,6</sup>. Since the phosphorylation of spectrin in the intact cell does not require cyclic AMP, we isolated the cyclic AMP-independent kinase, following the method of Hosey and Tao<sup>7</sup>, and tested its ability to phosphorylate spectrin in solution. Ghosts were prepared from human erythrocytes, not more than 1-d-old, by the procedure of Hanahan *et al.*<sup>8</sup>. Spectrin was extracted from water-washed ghosts either by agitation for 1 h at 37 °C or by a 24-h dialysis in the cold, and purified by gel filtration<sup>9</sup>. In the presence of  $\gamma$ -labelled <sup>32</sup>P-ATP and kinase, about 0.45 g atom of phosphorus per mol of spectrin was incorporated (Fig. 1a). Autoradiography of this material after separation on sodium dodecyl sulphate (SDS)-acrylamide gels confirms that the phosphate is located exclusively on the small subunit (Fig. 1b) as in endogenously phosphorylated spectrin<sup>1,3</sup>.

The effect of this phosphorylation on the spectrin-induced polymerisation of actin is dramatic (Fig. 2). A very rapid rise in viscosity is observed in conditions in which actin itself remains monomeric. The increase occurs without a detectable lag phase, and the reduced viscosity reaches a final level of 0.9–1.2 ml mg<sup>-1</sup> at an actin concentration of 0.5–0.6 mg ml<sup>-1</sup>, which is close to that achieved in the salt-induced polymerisation of actin alone. Phosphorylated spectrin containing about 0.45 g atom phosphorus per mol of protein produced a partial rise time 30 to 100 times that given by normally prepared spectrin. It was shown previously that the increase in viscosity is accompanied by the appearance of bundles of thin filaments composed principally of actin<sup>4</sup>, as well as some spectrin. We have now found that it is the phosphorylated form that is preferentially associated with the polymerised actin: thus in two experiments, the specific activity of <sup>32</sup>P-labelled spectrin carried down with the actin on centrifugation was more than 50 times that remaining in the supernatant.

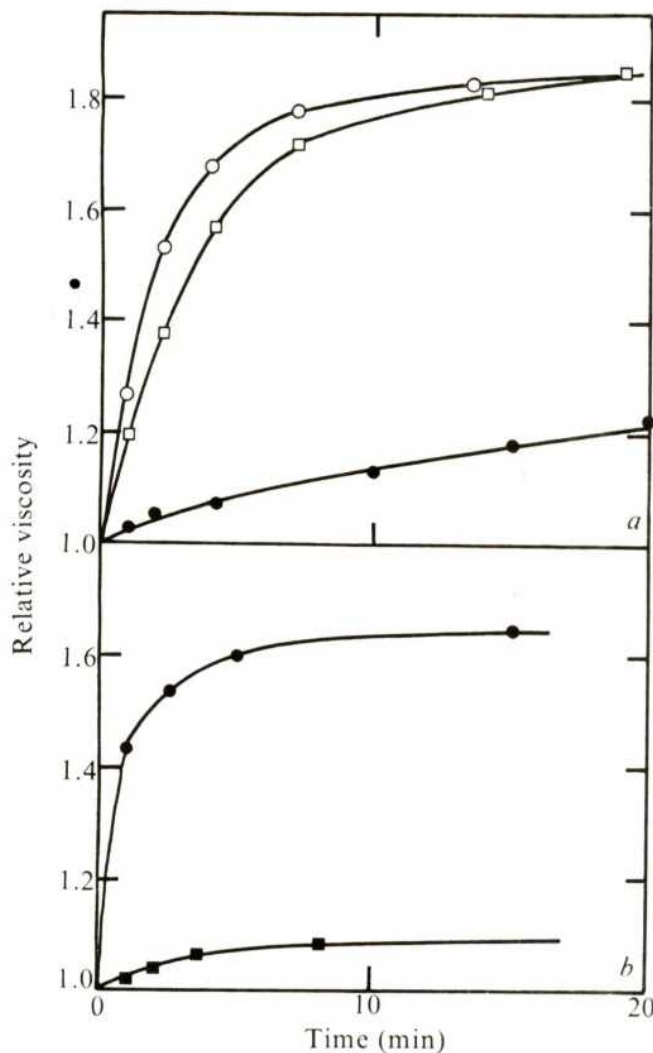
Treatment of the spectrin with bacterial alkaline phosphatase causes a loss of the phosphate group and almost entirely eliminates the capacity to induce actin polymerisation. That this is not a consequence of damage by endogenous proteolytic activity is shown by its reversibility: if kinase is added after the phosphatase, phosphorylation

occurs and polymerisation of actin ensues as before (Fig. 2). Relatively high concentrations of phosphatase are used in these experiments, spectrin being apparently a poor substrate for this bacterial enzyme. In the absence of phosphatase, storage of purified spectrin in buffer caused no



**Fig. 1** *a*, Phosphorylation of spectrin in solution by red cell membrane kinase. Crude spectrin extract was dephosphorylated with bacterial alkaline phosphatase (compare Fig. 2), and purified by column chromatography on Sephadex G200<sup>9</sup>; 100  $\mu$ l crude human erythrocyte membrane kinase<sup>7</sup> in 20mM Tris, 1mM dithiothreitol, pH 7.4, at 3 mg ml<sup>-1</sup> total protein concentration (estimated by colorimetric micro-Kjeldahl analysis<sup>10</sup>), was added to 0.6 mg dephosphorylated spectrin in 100mM sodium chloride, 10mM Tris, 5mM magnesium chloride, pH 7.4 at 4 °C. Phosphorylation was started by the addition of 1  $\mu$ l  $\gamma$ -<sup>32</sup>P-ATP of specific activity 1 mCi ml<sup>-1</sup> and the mixture incubated at 37 °C; 50- $\mu$ l aliquots were removed at intervals, spotted on to squares of Whatman No. 1 filter paper and air-dried. Labelled protein was fixed and excess radioactivity removed by washing in two changes of cold 5% trichloroacetic acid (TCA) for 15 min each time, followed by boiling for 15 min and a further cold TCA wash. The paper squares were oven-dried at 60 °C and samples solubilised for counting by adding 0.5 ml Soluene (Packard) and incubating overnight at 20 °C. Then 3 ml scintillation fluid containing 5.5 g Permablend (Packard) and 1 ml acetic acid per 1 toluene were added and the samples counted in a Nuclear Chicago liquid scintillation counter. The data are corrected for background counts. *b*, Autoradiograph of gel electrophoresis in SDS of membrane protein extract after phosphorylation (lower), and stained gel (upper), with densitometer traces. This shows that only spectrin has been sensibly phosphorylated, and that the <sup>32</sup>P is incorporated exclusively in the smaller subunit.





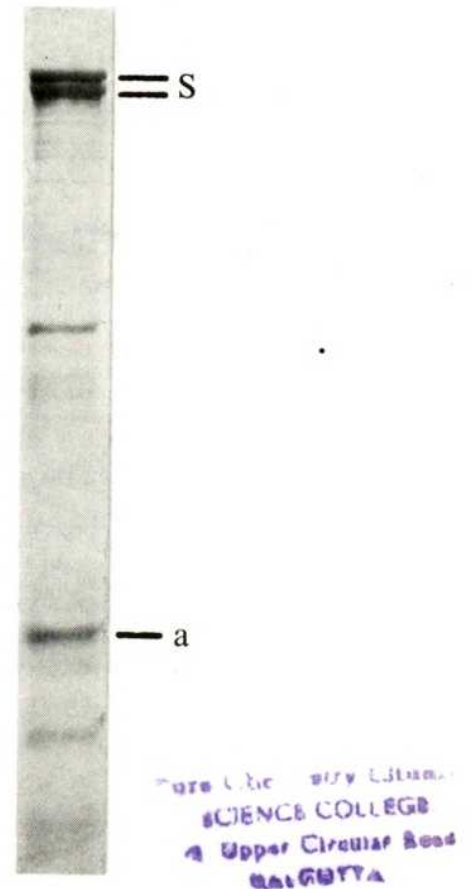
**Fig. 2** Effect of phosphorylation state of spectrin on polymerisation in actin-spectrin mixtures. Actin concentration,  $0.6 \text{ mg ml}^{-1}$  throughout. *a*, Effect of phosphorylation with red cell membrane kinase on time course of polymerisation: spectrin as prepared untreated (●), after treatment with kinase (○), and after serial exposure to alkaline phosphatase, and kinase (□). The spectrin concentration in (a) is  $0.04 \text{ mg ml}^{-1}$ . *b*, Effect of dephosphorylation with alkaline phosphatase on time course of polymerisation: spectrin as prepared, untreated (●), and after treatment with phosphatase (■). Spectrin concentration in (b),  $0.4 \text{ mg ml}^{-1}$ . The spectrin was prepared by extraction at  $37^\circ \text{C}$  for 1 h<sup>12</sup>, and purified by column chromatography on Sephadex G200<sup>®</sup>. The G-actin was prepared from rabbit skeletal muscle by the method of Spudich and Watt<sup>13</sup>. The procedure for viscometry and the solvent conditions were as described previously<sup>1</sup>—Ostwald capillary viscometer with water flow time of 40 s, maintained at  $30^\circ \text{C}$ ; solvent was 10 mM sodium phosphate, 0.1 mM calcium chloride, 0.2 mM ATP, 0.2 mM dithiothreitol, pH 8.2. Kinase-catalysed phosphorylation of spectrin was allowed to proceed for 30 min in the presence of 1 mM ATP (see Fig. 1). Spectrin was dephosphorylated by the addition of 0.25 mg bacterial alkaline phosphatase (Worthington) per mg spectrin in 10 mM Tris, 140 mM potassium chloride, 20 mM sodium chloride, pH 7.4, followed by incubation at  $37^\circ \text{C}$  for 2 h. After treatment with the enzymes the spectrin was dialysed in the cold into the buffer used for viscometry. Note the 10-fold difference in spectrin concentration between (a) and (b) to allow comparison of untreated with both kinase and phosphatase-treated material.

perceptible loss of phosphorus in periods of up to some days. Treatment of actin alone, with either enzyme, had no effect on its polymerisation properties.

In our previous study<sup>4</sup> we found that the ability to cause actin polymerisation was shown only by spectrin prepared from fresh red blood cells, and even then the response showed some variability. By contrast, the spectrin phosphorylated as described above induced polymerisation in a

consistent and reproducible fashion. Indeed, inactive preparations of spectrin, such as those prepared from stored blood, have been reactivated by phosphorylation. It seems, therefore, that the previously observed variability was due to a failure to control the phosphorylation state of spectrin.

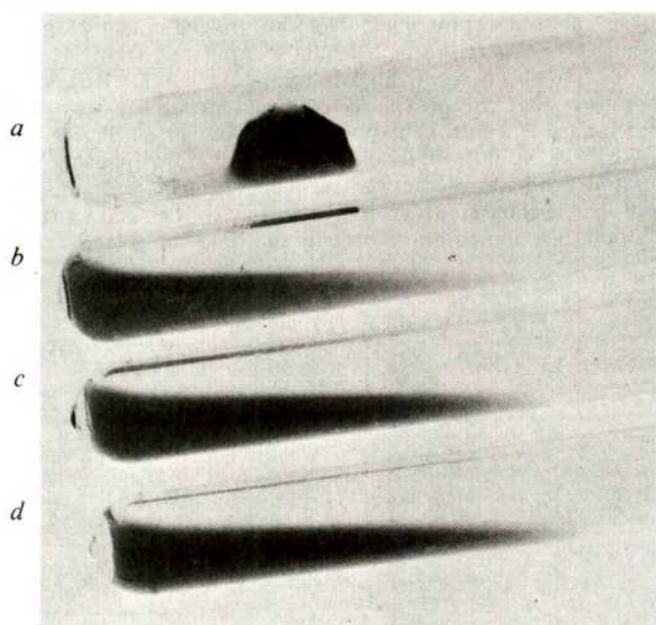
We have attempted to establish whether the phosphorylation-dependent interaction between spectrin and muscle actin has a counterpart in the erythrocyte. It is known that the bulk of the membrane protein and lipid components can



**Fig. 3** Polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulphate (SDS), of cytoskeletal extract from human erythrocyte membranes. Washed ghosts were prepared by the method of Dodge *et al.*<sup>8</sup>, but with inclusion in the lysis buffer of 0.2 mM dithiothreitol, 0.2 mM ATP calcium chloride; 5 ml of the undiluted washed ghost pellet were added to 20 ml 1% Triton X-100, containing 25 mM Tris, 0.2 mM ATP, 0.2 mM calcium chloride, pH 7.4, mixed gently and incubated at  $37^\circ \text{C}$  for 30 min to maximise extraction of lipid. The pellet obtained after centrifugation at  $80,000g$  for 3 h was solubilised by heating at  $100^\circ \text{C}$  for 5 min in 0.125 M Tris, 1% SDS, pH 6.8, and subjected to electrophoresis in an 8% polyacrylamide gel using a discontinuous Tris-glycine buffer system containing 0.1% SDS<sup>19</sup>. The gels were stained with 0.05% Coomassie brilliant blue R in methanol-acetic acid-water (5:1:5 v/v), and de-stained in 10% acetic acid.

be extracted from human erythrocyte ghosts with the non-ionic detergent Triton X-100 (ref. 10). Such extraction leads to what in the electron microscope appears as a fibrous network, retaining the outline of the shape of the ghosts<sup>10</sup>. When this residue is examined by SDS-acrylamide gel electrophoresis, the major components are found to be actin and spectrin (Fig. 3). The Triton-extracted ghost residues may be dispersed by gentle homogenisation in buffer to give a mobile suspension with a total protein concentration of  $20 \text{ mg ml}^{-1}$ . When the kinase preparation is added to this suspension at  $37^\circ \text{C}$ , a stiff gel is rapidly formed (Fig. 4). This phenomenon not only depends on





**Fig. 4** Phosphorylation-dependent gelation of cytoskeletal spectrin-actin residue. Pellets prepared from Triton X-100 extract of ghosts (see Fig. 3) containing 20 mg total protein (equivalent to 10 ml ghost suspension), as estimated by Folin-Ciocalteu analysis, were homogenised gently in 10 mM Tris, 150 mM potassium chloride, 5 mM magnesium chloride, 1 mM ATP, pH 7.4, to give a total volume of 1 ml. The tubes contain this mixture; *a*, after addition of 3 mg crude red cell membrane kinase; *b*, untreated; *c*, after addition of 1 mg DNAase I (Sigma), followed by incubation at 37 °C for 30 min, and addition of 3 mg kinase; *d*, as (*c*) but in the absence of kinase. All mixtures were then maintained at 37 °C for 30 min. The gelation observed in (*a*) occurred within 5 min.

phosphorylation by the kinase, but also requires the presence of the actin: this is demonstrated by the introduction of the specific actin-sequestering protein, deoxyribonuclease I<sup>11,12</sup>. If this is added to a concentration about equimolar to that of actin, phosphorylation then fails to induce any formation of gel, or any other macroscopically observable effect (Fig. 4).

The formation of the gel by the cytoskeletal extract suggests that phosphorylation leads to a cross-linked complex of spectrin and actin; the inhibitory effect of deoxyribonuclease indicates that actin is directly implicated in the change of state, but does not prove that this involves a polymerisation, rather than an association between spectrin and actin monomers. It may be relevant in this context to note that muscle and erythrocyte actin are not identical, and may be distinguished by two-dimensional separation on a polyacrylamide gel<sup>13</sup>.

The results described here extend our earlier inference of a specific functional relationship between spectrin and actin in the erythrocyte membrane<sup>4</sup>. Although spectrin has so far been identified only in the red blood cell<sup>14</sup>, gelation phenomena have been observed in systems from several other kinds of cells, involving actin and high molecular weight proteins (see ref. 15 for review). These are of great interest because they could reflect mechanisms by which actin polymerisation is controlled in the cell. The requirement for ATP by these gelation reactions may be an indication that, as in the system described here, phosphorylation of one of the protein components is mandatory.

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1. Birchmeier, W. & Singer, S. J. *J. Cell Biol.* **73**, 647–659 (1977).
2. Makoto, N., Nakao, T. & Yamazoe, S. *Nature* **187**, 945–946 (1960).
3. Gratzer, W. B. & Beaven, G. H. *Eur. J. Biochem.* **58**, 403–409 (1975).
4. Pinder, J. C., Bray, D. & Gratzer, W. B. *Nature* **258**, 765–766 (1975).
5. Rubin, C. S., Erlichman, J. & Rosen, O. M. *J. biol. Chem.* **247**, 6135–6139 (1972).
6. Avruch, J. & Fairbanks, G. *Biochemistry* **13**, 5507–5514 (1974).
7. Hosey, M. M. & Tao, M. *Biochim. biophys. Acta* **482**, 348–357 (1977).
8. Dodge, J. T., Mitchell, C. & Hanahan, D. J. *Archs Biochem.* **100**, 119–130 (1963).
9. Pinder, J. C., Tidmarsh, S. & Gratzer, W. B. *Archs Biochem.* **172**, 654–660 (1976).
10. Yu, J., Fischman, D. A. & Steck, T. L. *J. supramol. Struct.* **1**, 233–248 (1973).
11. Lazarides, E. & Lindberg, U. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4742–4746 (1974).
12. Hitchcock, S. E., Carlsson, L. & Lindberg, U. *Cell* **7**, 531–542 (1976).
13. Garrels, J. I. & Gibson, W. *Cell* **9**, 793–805 (1976).
14. Hiller, G. & Weber, K. *Nature* **266**, 181–183 (1977).
15. Hitchcock, S. E. *J. Cell Biol.* **74**, 1–15 (1977).
16. Jaenicke, L. *Analyt. Biochem.* **61**, 623–627 (1974).
17. Marchesi, V. T. *Meth. Enzym.* **32**, 275–277 (1974).
18. Spudich, J. A. & Watt, S. J. *J. biol. Chem.* **246**, 4866–4871 (1971).
19. Laemmli, U. K. *Nature* **227**, 680–685 (1970).

## Formation of branched DNA structures by *Xenopus laevis* oocyte extract

We have reported that a soluble cell-free extract from stage 6 oocytes of *Xenopus laevis* can act on supercoiled SV40 DNA to produce molecules with a single-strand scission, linear molecules of full length, shorter fragments and various forms of complex DNA<sup>1</sup>. The complex DNA consisted of various structures, such as figure-of-eight dimers, catenated dimers, circular dimers, catenated trimers, late Cairns' structures, complex multimers and circular monomers with tails. The few late Cairns' structures observed could have been due to replication, but it is possible that they and the other forms of complex DNA resulted from recombination among SV40 DNA molecules. We thought that fractionation of the extract should produce fractions lacking some of the activities necessary to produce complex DNA, so that intermediate structures would accumulate. We now report that some fractions of the extract produce a high percentage of branched DNA structures from SV40 DNA, and we describe the requirements and characteristics of this reaction. We also discuss the possibility that branched DNA is an intermediate in the formation of complex DNA.

The extract was fractionated and prepared as described in the legend to Fig. 1. The fractions eluting from the phosphocellulose column at 0.2–0.25 M KCl produced nicked circles, linear molecules and branched DNA. The branched DNA was very varied in form (circular and linear), length, number and arrangement of branches (Fig. 1). The length of the molecules in the branched structures, in view of the length of the SV40 DNA, indicated that more than one molecule was involved.

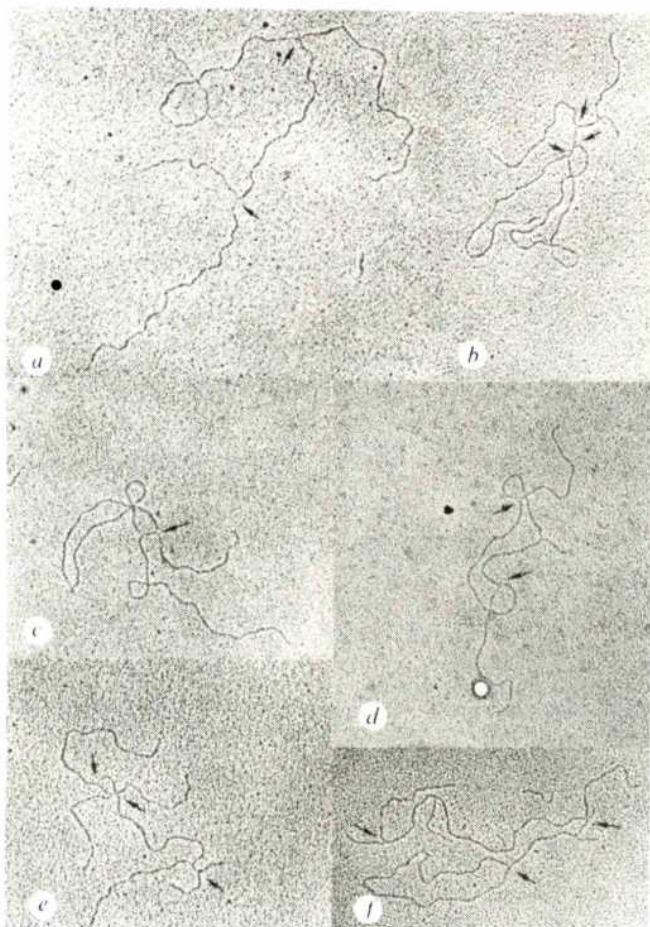
**Table 1** Effect of temperature and time of incubation on the formation of branched structures

Temperature (°C)	Time (min)	% Branched structures	No. molecules scored
30	60	3.6	1,110
	120	11.8	1,180
37	60	8.9	1,120

Supercoiled SV40 DNA at 14 µg ml<sup>-1</sup> was incubated with the phosphocellulose fraction in the conditions indicated in Fig. 1.

There were often several branches in one structure; because of the high dilution used for the electron microscopy preparation, these multiple-branched forms cannot be due to random association. The structures formed without added deoxytriphosphates, and it was highly unlikely that any endogenous deoxytriphosphates remained in the conditions used. Thus branched DNA structures were probably formed in the absence of DNA synthesis.





**Fig. 1** Electron micrographs of branched structures produced by incubating supercoiled SV40 DNA with a phosphocellulose fraction. Supercoiled SV40 DNA was prepared as previously described<sup>1</sup>. To prepare the phosphocellulose fractions, 20 ml of collagenase-treated stage 6 oocytes (about 14,000 cells) were used. All operations were carried out at 0–4 °C. The oocytes were homogenised in a Dounce homogeniser with 80 ml of TEMG buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1.4 mercapto-ethanol, 20% (w/v) glycerol). The homogenate was centrifuged for 15 min at 8,000g and the supernatant, after removal of the floating lipid layer, was centrifuged in a Beckman 50 Ti rotor at 40,000 r.p.m. for 60 min. The high-speed supernatant was brought to 60% saturation with ammonium sulphate in the cold. The precipitate was collected by centrifugation, dissolved in 15–20 ml of TEMG buffer, and dialysed exhaustively against TEMG. The dialysate was centrifuged for 30 min at 30,000g to remove insoluble proteins and applied to a DEAE-cellulose column (Whatman DE-52) (3 × 20 cm) equilibrated with TEMG buffer. The column was washed with 3 volumes of buffer and eluted with TEMG containing 0.2 M KCl. All the fractions containing proteins, as detected by absorbance at 280 nm, were pooled (50–60 ml) and applied to a phosphocellulose column (1 × 5 cm) equilibrated with TEMG buffer. The column was washed with 3 volumes of buffer and subsequently eluted with 40 ml of a linear gradient of 0–1.0 M KCl in TEMG containing bovine serum albumin (0.5 mg ml<sup>-1</sup>). Fractions (1.5 ml) were collected, dialysed against TEMG buffer, divided in aliquots and stored at –70 °C. The dialysed fractions were assayed for the formation of branched structures in a reaction mixture containing in a volume of 50 µl, 50 mM Tris-HCl, pH 7.5, 6 mM Mg Cl<sub>2</sub>, 6 mM dithiothreitol, 0.1–0.5 µg of SV40 DNA and 25 µl of the phosphocellulose gradient fractions. After incubation for 60 min at 30 °C, the reaction was stopped by addition of sodium dodecyl sulphate and EDTA to final concentrations of 1% and 15 mM respectively. Distilled water (50 µl) was added to each reaction mixture and the DNA was extracted with phenol and ether and used directly for electron microscopy or after concentration by ethanol precipitation. Samples were prepared as previously described<sup>1</sup> (× 6,100). Arrows indicate branch points.

The formation of branched structures was absolutely dependent on the presence of magnesium. In the absence of ATP, we observed extensive degradation of the DNA. We therefore conclude that degradation is inhibited in the presence of ATP, but we could not show that ATP was involved in the formation of branched structures. Addition of the four deoxytriphosphates did not increase the proportion of branched structures, indicating further that DNA synthesis is not required.

Table 1 shows the effect of temperature and time of incubation on the formation of branched structures. The percentage of branched structures after 60 min of incubation at 37 °C was at least twice the percentage of branched structures formed at 30 °C. The reaction was time dependent: four times as many branched structures formed after 120 min at 30 °C as after 60 min. The generation of multiply-branched structures showed the same time dependence as did that of molecules with a single branch.

Because more than one molecule was involved in each branched structure the percentage of these forms should have increased with increasing DNA concentration. This was the case with concentrations up to 3 µg ml<sup>-1</sup>. At higher DNA concentrations the percentage of branched structures decreased, suggesting that, because the reaction was presumably catalysed by many factors, some of these became limiting at increasing DNA concentrations.

Supercoiled DNA was not required for the formation of branched structures (Table 2). We have observed that relaxed molecules, prepared by the action of *Xenopus laevis* DNA-relaxing enzyme<sup>1</sup>, are four times more efficient than the supercoiled form; both nicked circles with a single-strand scission and linear molecules react with approximately the same efficiency. We cannot explain the relatively low efficiency of supercoiled DNA. Table 2 shows a correlation among multiple branches in a single structure; when linear molecules were used as substrate, 20.5% of the 500 structures observed were

branched. An independent origin of each branch would have led to  $(0.205)^2 \times 500 = 21$  multiply-branched structures instead of the 46 we observed. Similar considerations apply when other molecular forms were used as substrate. The large number of multiply-branched structures formed suggests that two or more branches were generated as part of a single exchange event. Furthermore, multiple branches were often structurally very close together.

When linear SV40 DNA was used as substrate, a considerable number of branched structures had an H-like configuration. Figure 2a, b, c and d shows H-like and Y-like structures. When linear DNA was used, degradation due to exonuclease(s) activity in the fraction did not always enable us to recover intact molecules; therefore we could not map the cross bar of the H-like structures relative to the extremities.

The distribution of lengths of the regions between two

**Table 2** Summary of electron microscopic analysis of branched DNA structures

DNA	Branched (%)	Multiply-branched	
		Branched	× 100 No. molecules scored
Supercoiled	5.6	22.2	1,280
Open circles	23.8	47.6	500
Relaxed circles	20.6	46.1	500
Linear*	20.5	45.2	500

The various forms of SV40 DNA prepared as previously described<sup>1,4</sup> were incubated with the phosphocellulose fractions in the conditions indicated in Fig. 1 at 10 µg ml<sup>-1</sup> for 2 h at 30 °C. Samples were prepared for electron microscopy as previously described.

\*Various size fragments were produced when linear molecules were used as substrate. The percentage of branched molecules was estimated taking into account only molecules equal to or larger than SV40 full length size.



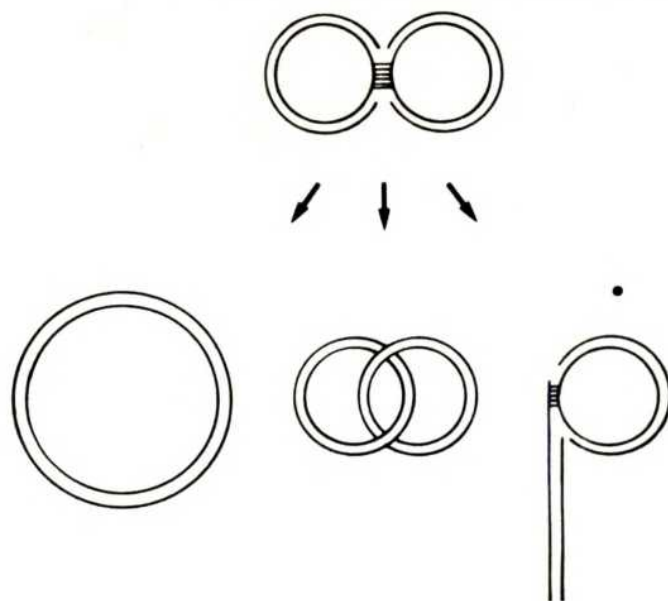
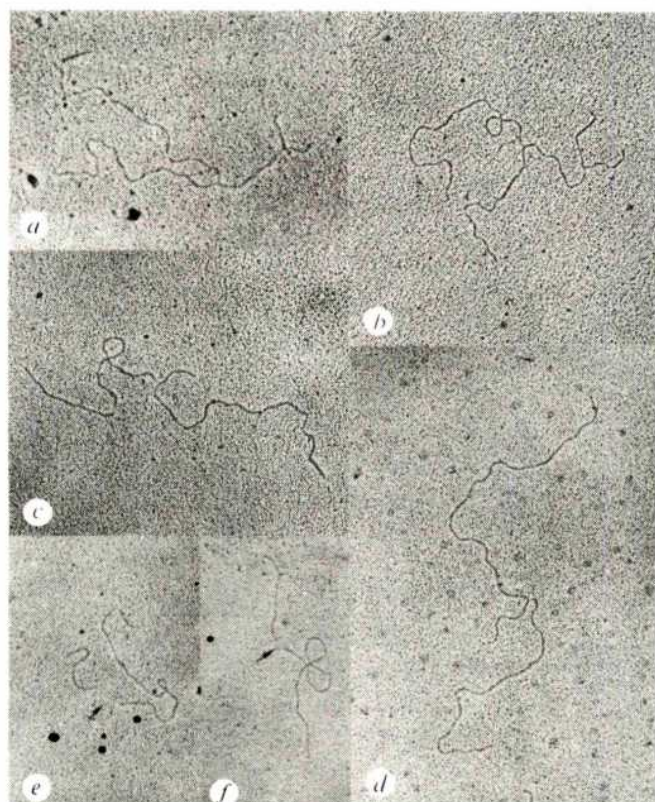
branched points were measured. The lengths varied from an apparent 0 to 0.5 fractional length, with an average of 0.15. Because SV40 chromosome contains about 5.1 Kb pairs, the average length of the region between two branched points corresponds to 765 nucleotides.

Models for the formation of branched DNA supported by evidence in the phage T4 and phage T7 experimental systems<sup>2,3</sup>, suggest that H-like and Y-like structures could be formed by a process involving an exonuclease which initiates hydrolysis from a nick produced by an endonuclease of the type which we have described<sup>4</sup>. An expanded single-strand region which paired with DNA homologous in that region, would yield H-like structures. This would occur if breaks are introduced on the opposite strands of two paired molecules. Gapped molecules were frequently observed in the electron micrographs of DNA incubated with the fractionated extract (Fig. 2e and f). Y-like structures could originate from the H-like structures through branch migration or from recombination of a terminal unpaired region with an internal site of a gapped molecule.

Branched DNA structures could well be intermediates in the formation of complex DNA. A recombinant structure of two circular gapped molecules indistinguishable by electron microscopy from a late Cairns' replicative intermediate, could generate the various forms of complex DNA (Fig. 3).

H-like structures cannot give rise, by simple maturation of intermediates, to two reciprocal recombinants; our cell-free system is not faithfully mimicking meiotic recombination.

**Fig. 2** Electron micrographs of branched structures obtained by incubating a phosphocellulose fraction with linear SV40 DNA produced by *EcoRI*. Supercoiled SV40 DNA was digested with *EcoRI* in a reaction mixture containing 100 mM Tris-HCl, pH 7.4, 50 mM NaCl, 5 mM MgCl<sub>2</sub> for 2 h at 37 °C. The reaction was stopped by addition of 1% sodium dodecyl sulphate and 15 mM EDTA and the linear DNA was purified on a 5–20% neutral sucrose gradient. The fractions corresponding to material sedimenting at 14.5S were pooled, precipitated with ethanol and stored at –20 °C. Sucrose gradients contained 0.1 M NaCl, 10 mM Tris-HCl, pH 7.5, 10 mM EDTA, pH 8.0. (x 6,100). a and b, H-type recombinants; c and d, Y-type recombinants; e and f, gapped molecules. Arrows indicate gaps.



**Fig. 3** Schematic representation of DNA recombination involving two gapped circles as intermediates. Dimers, catenated dimers and circles with tail are shown as possible final products.

In some systems recombination is brought about by a set of proteins operating in a complex<sup>5</sup>; if, however, some proteins are inactive or absent, structures which are not intermediates in the normal pathway could be formed.

It is possible that a type of recombination occurs in *Xenopus laevis* oocytes that produces a single recombinant chromosome. A circle of amplified ribosomal DNA could recombine with chromosomal DNA, and, by branch migration, roll along the chromosomal sequences, forming heteroduplex regions. Such structures could be responsible for rectification of sequences by gene conversion<sup>6</sup>, rectified chromosomal DNA being the final product.

The *in vivo* roles of the enzymes which produce the recombinant structures we have described remain unknown. But whatever the function of the enzymes, our system can produce heteroduplexes and can be used to investigate gene conversion in purified cell extracts.

After our manuscript was submitted for publication Benbow and Krauss reported the formation of recombinant DNA in a cell-free system of *Xenopus laevis* eggs<sup>7</sup>.

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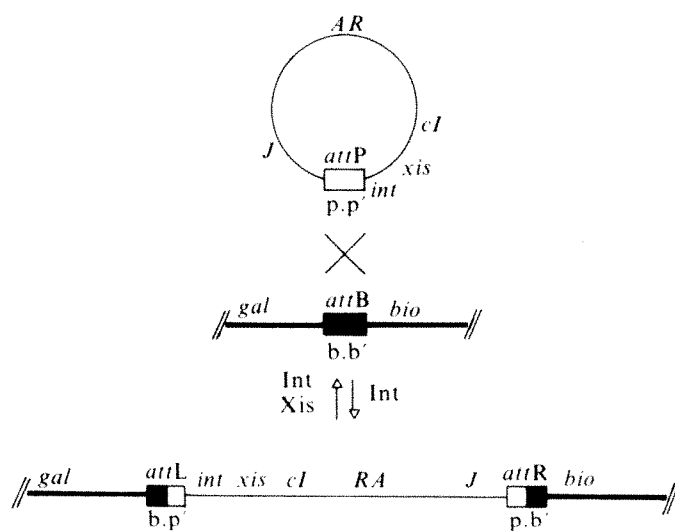
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- Gandini Attardi, D., Martini, G., Mattoccia, E. & Tocchini-Valentini, G. P. *Proc. natn. Acad. Sci. U.S.A.* 73, 554–558 (1976).
- Broker, T. R. & Lehman, I. R. *J. molec. Biol.* 60, 131–149 (1971).
- Tsujimoto, Y. & Ogawa, H. *J. molec. Biol.* 109, 423–436 (1977).
- Mattoccia, E., Gandini Attardi, D. & Tocchini-Valentini, G. P. *Proc. natn. Acad. Sci. U.S.A.* 73, 4551–4554 (1976).
- Mosig, G., Dannenberg, R. & Breschkin, A. *Indian J. Microbiol.* 15, 145–160 (1975).
- Tartof, K. D. *A. Rev. Genet.* 9, 355–385 (1975).
- Benbow, R. M. & Krauss, M. R. *Cell* 12, 191–204 (1977).

## Nucleotide sequence of the attachment site of coliphage lambda

MANY bacteriophages and animal viruses, as well as DNA insertion elements and transposable antibiotic resistance factors, are capable of integrating their genomes into specific sites in the genome of their hosts, giving rise to a range of important biological phenomena<sup>1-3</sup>. This site-specific recombination reaction has been studied most extensively in bacteriophage  $\lambda$  (ref. 3). During the process of lysogenisation the circularised phage DNA molecule is integrated into the *Escherichia coli* chromosome by reciprocal exchange between a specific site in the phage DNA molecule and a specific site in the *E. coli* chromosome between the *gal* and *bio* operons (Fig. 1). These sites are known as attachment sites (*att*). The reaction is catalysed by integrase, the product of the *int* gene<sup>4,5</sup>. Excision of the prophage by site-specific recombination between the prophage end attachment sites require the action of the product of the *xis* gene as well as that of the integrase<sup>6,7</sup>. In order to understand the mechanism of site-specific recombination it is necessary to study the DNA-protein interactions involved. Progress has recently been made in purification and study of the integrase<sup>8-10</sup>, and *in vitro* recombination systems have been developed<sup>11,12</sup>. We report here an important step in our comprehension of the reaction at the molecular level, the determination of the DNA sequence of the  $\lambda$  attachment site.

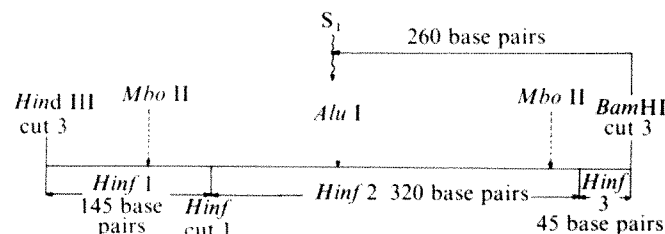
The middle of the attachment site was mapped within *Hinf*I fragment 2, the central *Hinf*I fragment in the region between *Hind*III cut 3<sup>13</sup> and *Bam*HI cut 3<sup>14</sup>, (Fig. 2), by Schreier *et al.*<sup>15</sup>.



**Fig. 1** Integration and excision of bacteriophage  $\lambda$ . Light lines represent the phage and prophage chromosome, heavy lines the bacterial chromosome. Rectangles represent the attachment sites. Genetic and physical data<sup>3</sup> show that each attachment site is made up of nonhomologous 'recognition elements' bracketing a central crossover point or region. Thus the phage attachment site, attP, is shown formally as p.p', the bacterial attB as b.b'. Distances between markers are arbitrary.

We have therefore determined the nucleotide sequence of *Hinf*I fragment 2 using the methods of Sanger and Coulson<sup>16</sup> and Maxam and Gilbert<sup>17</sup>. Using *Hinf*I fragment 1 (Fig. 2) as a primer for incorporation with DNA polymerase I on a  $\lambda$  r-strand template the plus and minus method gave a sequence extending from 14 to 230 base pairs from *Hinf*I cut 1 to the right, and using *Hinf*I fragment 3 on a  $\lambda$  l-strand template we obtained a sequence extending from 300 to 210 base pairs from *Hinf*I cut 1 to the right.

The end sequences (base pairs 3-45 at the left end and 246-317 at the right end) were determined by separating the strands of 5' end-labelled *Hinf*I fragment 2 and applying the method of Maxam and Gilbert<sup>17</sup>. Results obtained by the two methods agreed precisely. Figures 3 and 4 show plus and minus sequencing gels illustrating the central portion and the right half of att $\lambda$ . The sequence that we have determined is shown in Fig. 5. This sequence differs from



**Fig. 2** Physical map of the *Hind*III cut 3-*Bam*HI cut 3 fragment containing the  $\lambda$  attachment site. The sizes of *Hinf*I fragments 1 and 3 are given in base pairs, and are based on the data of Schreier *et al.*<sup>15</sup> and on the position of the labelled fragments in plus-minus sequencing gels when excess primer was used. The size of *Hinf*I fragment 2 is that determined by sequence analysis and the *Mbo*II cuts are placed accordingly. The solid vertical arrow above the *Alu*I site marks the position of the crossover point or left end of a common core (see text) as defined by the *S*<sub>1</sub> end of the *S*<sub>1</sub>-*Bam*HI fragment<sup>15</sup>. The map positions of the *Hind*III and *Bam*HI cuts are taken from references 13 and 14.

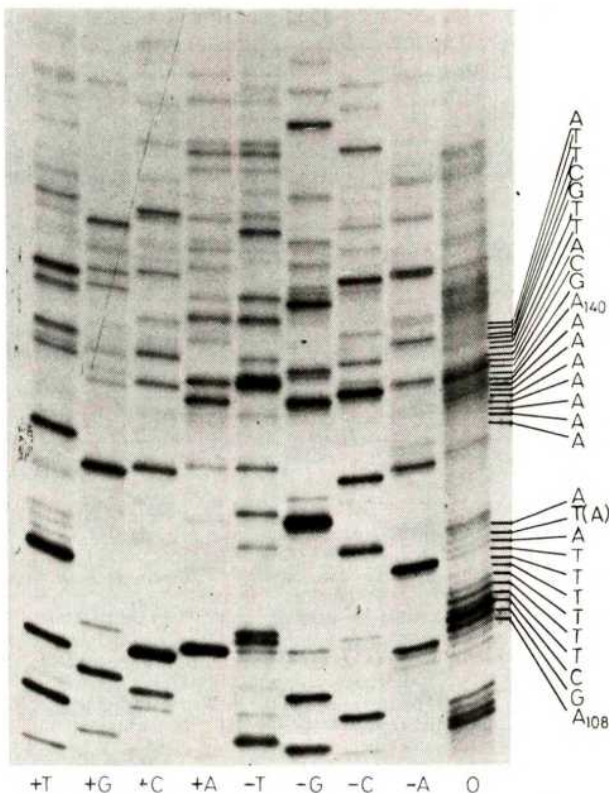
that determined independently by Landy and Ross<sup>26</sup> only in having A in the l-strand in positions 134, 151 and 179, where they give T.

We can locate the crossover site within the attachment sequence at  $105 \pm 10$  base pairs to the right of *Hinf*I cut 1. Schreier *et al.*<sup>15</sup> used the fact that the junction of phage and bacterial DNA in hybrid prophage end attachment sites corresponds to the position of the crossover site. Transducing phages such as  $\lambda$  *pgal*<sub>8</sub> carry a prophage end att, so that digestion of heteroduplex molecules between transducing phages and wildtype  $\lambda$  with the single-strand-specific nuclease *S*<sub>1</sub> and a restriction endonuclease leaves a fragment with one end at the crossover site and the other at a known restriction site. The distance of the crossover site from the restriction site was determined by comparing the mobility of these fragments with the mobility of standard  $\Phi$ X 174 fragments in polyacrylamide gels. The size of the relevant *S*<sub>1</sub>-*Bam*HI fragment from  $\lambda/\lambda$  *pgal*<sub>8</sub> heteroduplex molecules is 260 base pairs both in the native and denatured state. Thus the crossover site maps 260 base pairs left of *Bam*HI cut 3<sup>15</sup>. These considerations apply to the simplest model of site-specific recombination, where the recombination event takes place at a single internucleotide position<sup>20</sup>. An alternative model of att structure<sup>21,22</sup> proposes that recombination does not occur at a single site but by means of staggered nicks left and right of a small central region of homology common to phage and bacterial att. In this case the left end of the common homologous region maps 260 base pairs left of *Bam*HI cut 3 since the common homologous region would be included within the *S*<sub>1</sub> fragment.

In Fig. 5 the position of the crossover site is given with respect to the nearest *Hinf*I cuts, which depends on measuring the size of the three *Hinf*I fragments shown in Fig. 2. *Hinf*I fragments 1 and 3 are consistently 145 and 45 base pairs long respectively from their mobility as native and denatured fragments. The sequence data given here define the length of *Hinf*I fragment 2 as 320 base pairs. Therefore the crossover site is  $260 - 45 = 215$  base pairs left of *Hinf*I cut 2 (Fig. 2), and  $320 - 215 = 105$  base pairs to the right of *Hinf*I cut 1. The error of this determination is  $\pm 10$  base pairs.

The results of previous investigations<sup>22-25</sup> suggest that the attachment site consists of a central small crossover region flanked by 'recognition regions' that are not homologous with one another. Our sequence data, and that of Landy and Ross<sup>26</sup>, fit in with this. Landy and Ross<sup>26</sup> find that the sequence from base





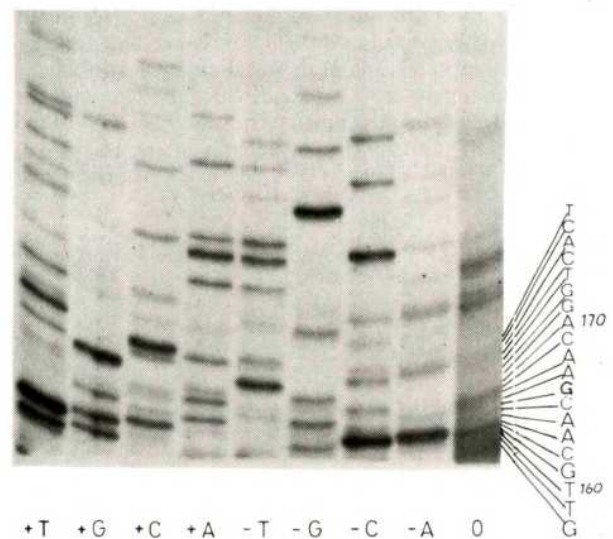
**Fig. 3** Autoradiograph of a plus and minus sequencing gel showing the important central region of the sequence. The gel shows the plus and minus pattern from base pair 95 to 195 to the right of *Hinf*I cut 1, produced by priming with *Hinf*I fragment 1 on a  $\lambda$  r-strand template. The source of DNA fragments was the col 1 based plasmid pMG 1409 containing the  $\lambda$  RIC DNA fragment, as described by Schreier *et al.*<sup>15</sup>. Plasmid purification and restriction enzyme digestion have been described previously<sup>15</sup>. *Bam*HI was purified from *Bacillus amyloliquefaciens* as described by Wilson and Young<sup>18</sup>. *Hind* III was purchased from Biolabs Inc. and *Hinf*I was a gift of A. Waltz and V. Pirotta. Fragments were purified from preparative polyacrylamide gels by crushing the gel, extracting three times for 12 h in 20 mM Tris pH 7.5, 0.1 mM EDTA, 0.1 M NaCl and precipitating with ethanol.  $\lambda$  single strands were prepared from  $\lambda$  c1857 S7 phage preparations according to Hradecna and Szybalski<sup>19</sup>. Annealing of template and primer, incorporation of labelled triphosphate ( $\alpha$   $^{32}$ P]dATP, New England Nuclear) and the plus and minus reactions were carried out according to Sanger and Coulson<sup>16</sup> with minor modifications. T4 polymerase was a gift of R. Kamen. To achieve reliable labelling of up to 250 bases incorporation was performed at 22 °C using the DNA polymerase I Klenow fragment (Boehringer, Mannheim), and 8% gels were used to analyse regions beyond 140 bases from the priming point. In the gel shown here each sample was further digested with *Mbo*II as well as *Hinf*I after the plus and minus treatment in order to cut *Hinf*I fragment 1 into smaller pieces. Otherwise the primer runs at position 143-146 making this part of the gel uninterpretable. In this gel the minus system does not show the T in position 118, so that we have labelled the gel T (A). This base is, however, clearly represented in three other gels covering this region.

pairs 108 to 124 in Fig. 5 is also present in the bacterial attachment sequence. Thus our location of the end of the  $S_1$ -*Bam*HI fragment corresponds well with the position of the left end of the common sequence. The sequences left and right of this region are completely different from one another.

The outer boundaries of *att* cannot as yet be defined. But, the sequences left and right of the crossover region although different in sequence are both regions of very high A + T content compared to the  $\lambda$  DNA molecule as a whole<sup>27</sup>. Regions of high A + T content are more susceptible to denaturation, particularly in supercoiled molecules<sup>28</sup> which have been shown to be the preferred substrate for integrative recombination of  $\lambda$ <sup>29,30</sup>. It is thus possible that these sequences have a physical role in site-specific recombination, by reducing the stability of the helix and facilitating local denaturation. This would explain the fact that a

number of point mutations in *att* have been found<sup>22,26</sup>, but all behave as if located in the central common region and none as if in the left or right recognition elements; single base changes would have little effect if the sequence were primarily important because of its A + T content. If this were so, it seems reasonable to propose that the right recognition element P' ends around base pair 200 in Fig. 5, since beyond base pair 200 the A + T content drops to the average  $\lambda$  level.

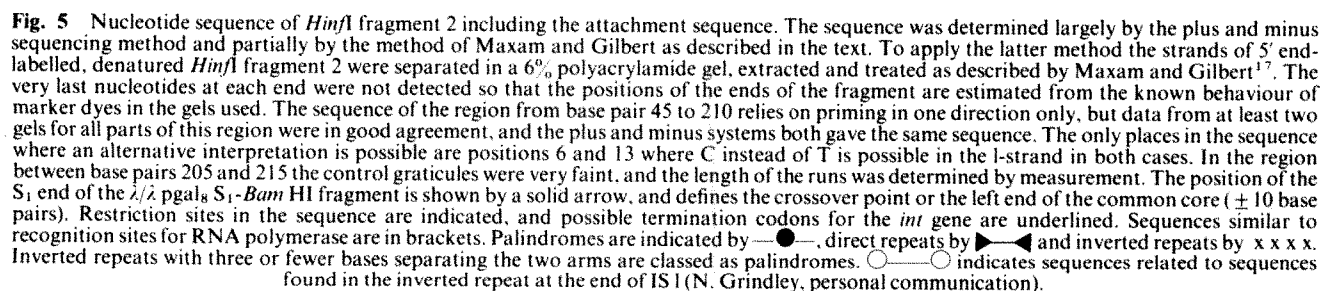
Since some *int* amber mutants do not recombine with a deletion of the attachment site so small as not to be detectable in the electron microscope<sup>10,25</sup>, the C-terminal end of the *Int* protein must be within 50 base pairs of the border of *att*. In the right half of the sequence given here there is a long open phase ending with a double stop at base pairs 199-194, another double stop signal being in phase at base pairs 178-173. The other two phases have stop signals for translation considerably further away from *att*. Therefore the last codon in the *int* gene is probably at base pairs 202-200. This corresponds precisely with the junction of a region of normal A + T content with the high A + T region around the crossover site. The resulting C-terminal protein sequence for integrase is given in Fig. 5. We are sequencing a C-terminal *int* amber mutant to test this interpretation.



**Fig. 4** Autoradiograph of an 8% polyacrylamide gel showing the plus and minus pattern from base pairs 165-220 and beyond after priming with *Hinf*I fragment 1 on a  $\lambda$  r-strand template, the experiment being performed as described in Fig. 3. The plus and minus method can be used to determine sequences of at least 200 base pairs in length from a single start point.

Other features of potential functional importance are shown in Fig. 5. There are three 12-base pair sequences (base pairs 39-50, 74-85 and 188-177) very similar to RNA polymerase recognition sequences found in known  $\lambda$  promoters. Two of them agree in 9 out of 12 bases with  $\lambda$ pr and three in 8 out of 12 bases with the general recognition sequence TGTGACAATTT obtained by comparison of 15 promoters in  $\lambda$ ,  $\Phi$ X 174, fd, T7, SV 40 and *E. coli*. Such recognition sequences are followed in promoter sequences after 12-14 base pairs by a sequence resembling TATPu-ATG<sup>31</sup>. Twelve bases after base pair 50 is the sequence TCTAATT. Transcription starting at either of the possible promoter sequences left of the crossover region might terminate at the mRNA termination sequence T<sub>6</sub>A (base pairs 111-117) in, or just right of, the crossover region. Small mRNA molecules originating in this region have been detected *in vitro* (*E. Rosen-vold* and *W. Szybalski*, personal communication). In and around the crossover region occur sequences that are also found close to the integration site of the DNA insertion element ISI (*N. Grindley*, personal communication). The sequence from base pair





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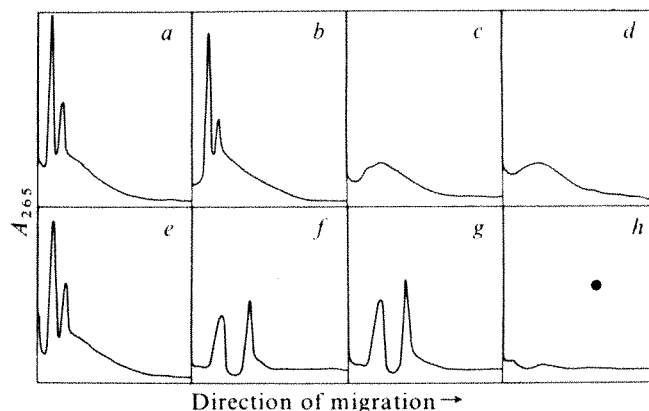
- Cohen, S. N. *Nature* **263**, 731-738 (1976).
- Battula, N. & Temin, H. M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 281-285 (1977).
- Gottesman, M. E. & Weisberg, R. A. in *The Bacteriophage Lambda* (ed. Hershey, A. D.) 113-138 (Cold Spring Harbor Laboratory, New York, 1971).
- Zissler, J. *Virology* **31**, 189 (1967).
- Gingery, R. & Echols, H. *Proc. natn. Acad. Sci. U.S.A.* **58**, 1507-1514 (1969).
- Guarneros, G. & Echols, H. *J. molec. Biol.* **47**, 565-574 (1970).
- Kaiser, A. D. & Masuda, Y. *J. molec. Biol.* **47**, 557-564 (1970).
- Nash, H. A. *Nature* **247**, 543-545 (1974).
- Kotewicz, M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 1511-1515 (1977).
- Enquist, L. W. & Weisberg, R. A. *J. molec. Biol.* **111**, 97-120 (1977).
- Gottesman, S. & Gottesman, M. E. *J. molec. Biol.* **81**, 489-499 (1975).
- Nash, H. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1072-1076 (1975).
- Murray, K. & Murray, N. E. *J. molec. Biol.* **98**, 551-564 (1975).
- Haggerty, D. M. & Schleif, R. F. *J. Virol.* **18**, 659-663 (1976).
- Schreier, P. H. *et al.* *Nature* **267**, 555-557 (1977).
- Sanger, F. & Coulson, A. R. *J. molec. Biol.* **94**, 441-448 (1975).
- Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560-564 (1977).
- Wilson, G. A. & Young, F. E. *J. molec. Biol.* **97**, 123-125 (1975).
- Hradecna, Z. & Szybalski, W. *Virology* **32**, 633-643 (1967).
- Dove, W. F. *J. molec. Biol.* **47**, 585-589 (1970).
- Signer, E. *Rev. Microbiol.* **22**, 451-488 (1968).
- Shulman, M. & Gottesman, M. E. *J. molec. Biol.* **81**, 461-482 (1973).
- Guerrini, F. *J. molec. Biol.* **46**, 523-542 (1969).
- Davis, R. W. & Parkinson, J. *J. molec. Biol.* **56**, 403-423 (1971).
- Shulman, M., Mizuuchi, K. & Gottesman, M. E. *Virology* **72**, 13-22 (1976).
- Landy, A. & Ross, W. *Science* **197**, 1147-1160 (1977).
- Davidson, N. & Szybalski, W. in *The Bacteriophage Lambda* (ed. Hershey, A. D.) 45-82 (Cold Spring Harbor Laboratory, New York, 1971).
- Botchan, P. *J. molec. Biol.* **105**, 161-176 (1976).
- Mizuuchi, K. & Nash, H. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3524-3528 (1976).
- Gellert, M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 4474-4478 (1976).
- Prinow, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 784-788 (1975).

## Plant viruses with circular single-stranded DNA

SMALL quasi-isometric particles, mostly occurring in pairs, have been found in, and purified from extracts of plants infected by maize streak<sup>1</sup>, beet curly top<sup>2</sup>, tomato golden mosaic<sup>3</sup>, euphorbia mosaic<sup>4</sup>, bean golden (yellow) mosaic<sup>5-6</sup>, cassava latent<sup>6</sup> and cassava brown streak viruses<sup>6</sup>. Individual particles are 15-20 nm in diameter—unusually small for a virus—and in electron micrographs many of the individual particles in the pairs have a five-sided outline in which the contiguous sides seem longer than the others. The pairs of particles of maize streak and cassava latent viruses have sedimentation coefficients of about 76S (refs 1 and 7) and preparations of each yield a single polypeptide species, estimated at about 28,000 and 34,000 daltons respectively<sup>7</sup>. Their nucleic acid can be resolved into two components by electrophoresis<sup>7</sup>, and in maize streak virus it has been identified tentatively as RNA on the basis of sensitivity to ribonuclease<sup>1</sup>. We now report, however, that the nucleic acids of cassava latent and maize streak viruses consist of single-stranded, predominantly circular DNA of molecular weight less than 10<sup>6</sup>.

Maize streak virus was purified as before<sup>1</sup> from maize plants infected by the leafhopper *Cicadulina mbila*. Cassava latent virus was purified from systemically infected leaves of *Nicotiana glauca* plants that were inoculated manually with sap. Some preparations were purified as before<sup>7</sup>, but in most purifications formaldehyde was omitted from the buffer used to resuspend the sediments of the first high-speed centrifugation. Viruses were propagated and purified at Nairobi, and nucleic acid was extracted there for infectivity tests. Nucleic acid for analysis was extracted at Invergowrie and examined by electron microscopy at Glasgow.

Electron microscopy showed that virus preparations contained many isometric particles of characteristic size, mostly in pairs. Maize streak virus particles seemed better preserved than those of cassava latent virus. When extracted<sup>17</sup> and analysed on 2.2% polyacrylamide gels in our standard electrophoresis buffer (0.036 M Tris, 0.03 M NaH<sub>2</sub>PO<sub>4</sub>, 0.001 M EDTA, pH 7.8), the nucleic acid of each virus usually gave two well resolved peaks (Fig. 1), but sometimes a single peak with a shoulder was obtained, as found before with that buffer<sup>7</sup>. There was usually more of the slower migrating component. Peaks were unaffected when samples were treated with briefly boiled RNase A before electrophoresis but were absent after



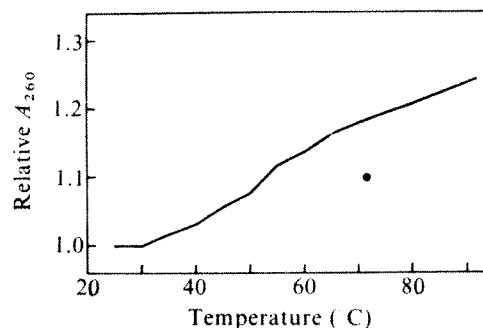
**Fig. 1** Effects of enzyme treatments on virus nucleic acid species separated by electrophoresis on 2.2% polyacrylamide gels. Nucleic acid (25-50 µg ml<sup>-1</sup>) was extracted using Pronase-sodium dodecyl sulphate<sup>17</sup> from cassava latent virus purified as described by Bock *et al.*<sup>7</sup> (a,e), cassava latent virus purified by the usual method (b-d), and maize streak virus (f-h). Treatments, for 30 min at 37 °C, were in 0.05 M Tris buffer, pH 7.5 (a-c and e-h) or in 0.01 M sodium acetate buffer, pH 5.0, containing 0.1 M sodium chloride (d). a,b,f, Buffer controls; c,h, DNase I (1 µg ml<sup>-1</sup> DN-EP, Sigma) + 3 mM MgCl<sub>2</sub>; d, S1 nuclease (500 U ml<sup>-1</sup> Type III, Sigma); e, g, RNase A (1 µg ml<sup>-1</sup>, bovine pancreatic, Type IA, Sigma, previously boiled for 1 min). After treatment, 4 volumes of ethanol were added to each sample. After resuspending the precipitates in standard electrophoresis buffer containing 8 M urea, the samples were heated for 15 min at 60 °C and electrophoresed for 3-3.5 h<sup>17</sup>. The gels were washed for 16 h in water and examined using an ultraviolet Scanner (Joyce Loeb).

treatment with DNase I (Fig. 1). Moreover they were unaffected by treating cassava latent virus nucleic acid with alkali (0.3 M NaOH for 8 h at 37 °C) before electrophoresis. The ability of cassava latent virus nucleic acid to infect *N. glauca* was destroyed by DNase but not RNase. Thus the nucleic acid of both viruses is DNA. The tentative identification of the nucleic acid of maize streak virus as RNA<sup>1</sup> can be attributed to the use of unboiled ribonuclease, presumably contaminated with DNase.

When nucleic acid of cassava latent virus was exposed to nuclease S1 before electrophoresis, neither of the characteristic nucleic acid species was found (Fig. 1), indicating that they are largely or entirely single-stranded. Resistance to the three nucleases was the same for nucleic acid from cassava latent virus purified with formaldehyde as when no formaldehyde was used.

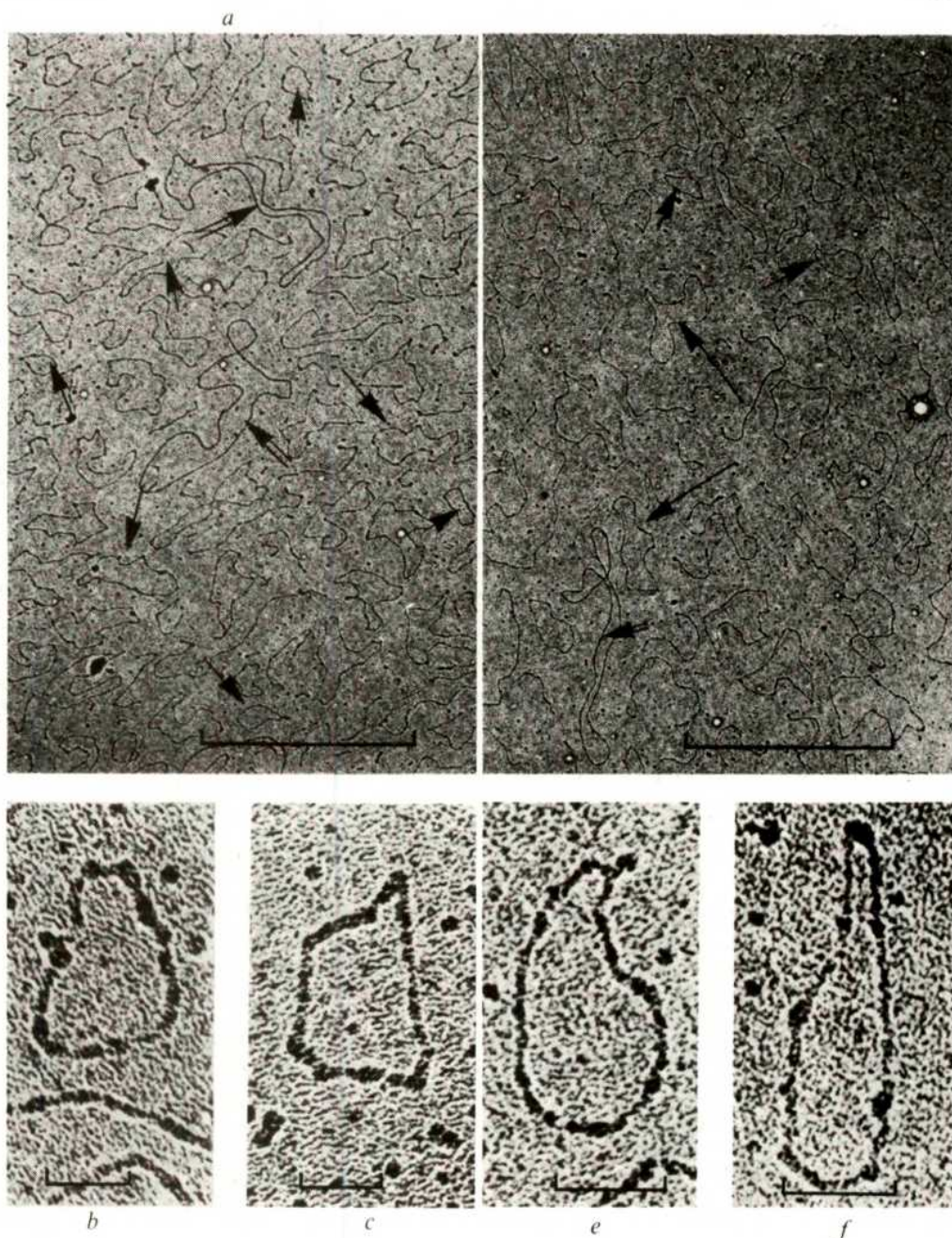
Further evidence that cassava latent virus nucleic acid is not substantially double-stranded came from plots of ultraviolet

**Fig. 2** Effect of temperature on ultraviolet absorption of cassava latent virus nucleic acid. Sample in 1.5 mM sodium chloride, 0.15 mM sodium citrate was heated at 0.5 °C per min. Readings are corrected for sample expansion.





**Fig. 3** Electron micrographs of nucleic acid molecules of maize streak (*a*, *b* and *c*), and cassava latent (*d*, *e* and *f*) viruses. The small circles in (*a*) and (*d*) are the plant virus nucleic acid molecules (short arrows), the middle-size circles are phage  $\Phi$ X174 strain am3 single-stranded DNA (long arrows) and the large circles are phage PM2 double-stranded DNA (double-tailed arrows). The bar represents 1  $\mu$ m. Individual molecules of maize streak virus nucleic acid are illustrated at higher magnification in (*b*) and (*c*) and those of cassava latent virus nucleic acid in (*e*) and (*f*). The bar represents 0.1  $\mu$ m. The nucleic acid was prepared for electron microscopy by the formamide modification of the protein film technique (ref. 12) from 50% (v/v) formamide, 0.1 M Tris-HCl, 0.01 M EDTA (pH 8.5) on to a hypophase of 20% (v/v) formamide, 0.01 M Tris-HCl, 0.001 M EDTA (pH 8.7). Micrographs were taken with a Siemens 101 electron microscope at 40 kV and a nominal magnification of 12,000 fold.



absorption at 260 nm in the range of 25°–92 °C (Fig. 2). There was no evidence of cooperative melting. Moreover when the nucleic acids were heated to 92 °C, cooled slowly to 25 °C and heated again to 90 °C, the final absorbance curve was essentially the same shape as before, indicating that no extensive double-stranded structures had formed on cooling, and excluding the possibility that some virus particles contain DNA-plus strands while a substantial proportion contain complementary minus strands instead, as with some parvoviruses<sup>9</sup>.

Electron microscopy of preparations of nucleic acid of either virus revealed two sorts of molecule (Figs 3 and 4). Small circular molecules predominated in most preparations but in some there were about as many linear as circular molecules and, in a few, linear molecules predominated. Linear molecules varied in size up to the length of the circular molecules (Fig. 4), suggesting that they are formed by nicking and partial degradation of the latter. In samples to which double-stranded circular DNA of phage PM2<sup>10</sup> and single-stranded circular DNA of phage  $\Phi$ X174 (strain am3)<sup>11</sup> were added, the plant virus DNA molecules

appeared single-stranded throughout.

Molecular weight was estimated from relative molecule lengths<sup>12</sup>. Comparison of modal lengths showed that the circular DNA of cassava latent virus was about 50% of the size of phage  $\Phi$ X174-DNA (strain am3;  $1.59 \times 10^6$  daltons<sup>11,13</sup>), and had a molecular weight of  $0.80 \pm 0.01 \times 10^6$  (Fig. 5). The figures for maize streak virus DNA were about 45% and  $0.71 \pm 0.01 \times 10^6$ .

Length-distribution diagrams of DNA molecules from each virus (Fig. 4) suggested that the two nucleic acid species detected by polyacrylamide gel electrophoresis are linear and circular molecules of the same length. To examine this further, the two species in a preparation of cassava latent virus DNA were separated by polyacrylamide gel electrophoresis for 13 h, and the nucleic acid was extracted from each band by steeping the disrupted gel slices for 4–5 h at 4 °C in 2 ml 50% (v/v) formamide containing 1.5 mM sodium chloride and 0.15 mM sodium citrate. After low-speed centrifugation to remove gel fragments, the nucleic acid was concentrated from the super-



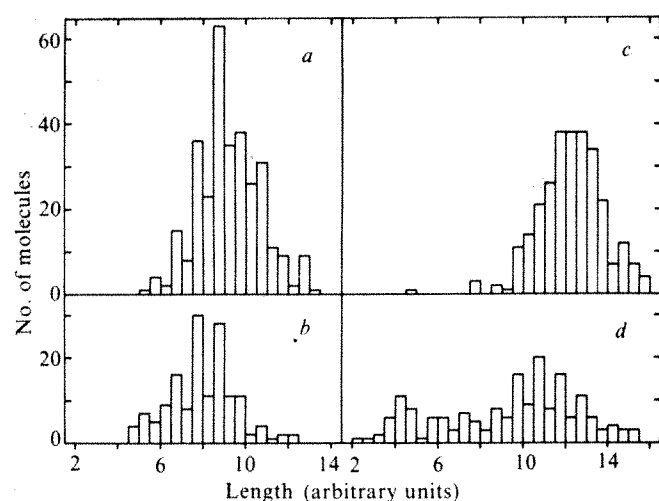


Fig. 4 Relative lengths of circular and linear molecules of virus DNA. *a*, Maize streak virus circular DNA (314 molecules); *b*, maize streak virus linear DNA (151 molecules); *c*, cassava latent virus circular DNA (279 molecules); *d*, cassava latent virus linear DNA (179 molecules). The molecules were measured with a map ruler from electron micrographs enlarged 25-fold.

nant fluid by precipitation with 4 volumes of ethanol, and the two samples were examined by electron microscopy. The sample of faster migrating component contained linear molecules only, many of similar length to the circular molecules. More than

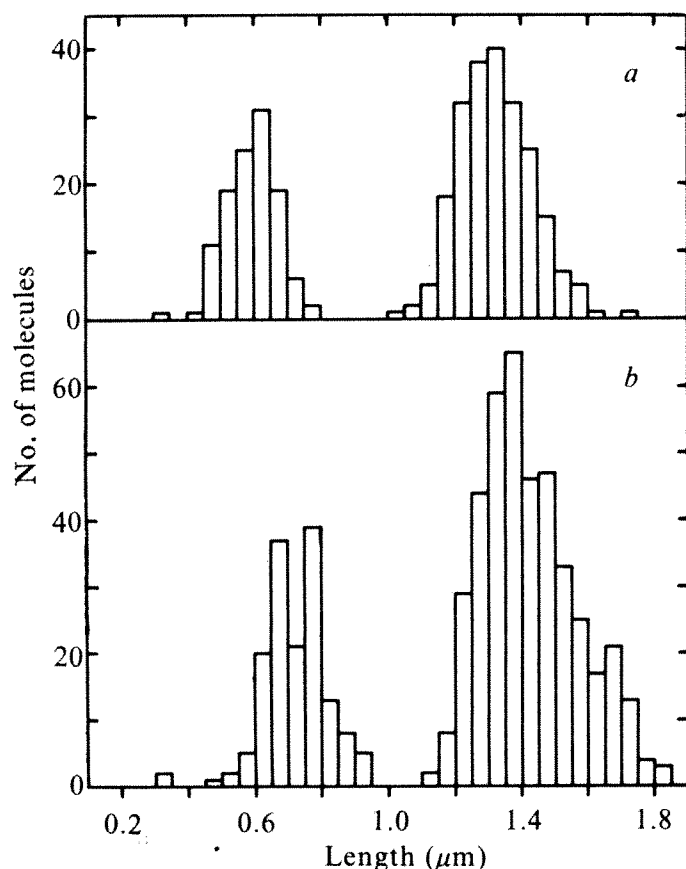


Fig. 5 Length-distribution diagrams for circular DNA molecules. *a*, Maize streak virus (115 molecules) and  $\Phi$ X174-am3 (222 molecules); *b*, cassava latent virus (153 molecules) and  $\Phi$ 174-am3 (416 molecules). Each pair of DNA samples was mixed and spread. The method of specimen preparation is described in Fig. 3. Lengths were measured with a computer-assisted planimetric device<sup>18</sup> from micrographs enlarged 10-fold, the circles of  $\Phi$ X174 single-stranded DNA being used as an internal standard.

80% of molecules in the sample of slower migrating component, however, were circular. We conclude that the circular and linear molecules are respectively the slower and faster migrating component in polyacrylamide gels.

Working with single-stranded DNA from SV40 virus, Dingman *et al.*<sup>14</sup> found that the relative speeds of electrophoretic migration of circular and linear molecules of the same length depended on the concentration of the gel. Hence we attribute differences in the separation of the two DNA species in our gels to small uncontrolled differences in concentration of different batches of gel.

It seems likely that the nucleic acids of other plant viruses with small paired particles will be found to have similar single-stranded DNA. Of such viruses, some have leafhopper vectors whereas others have whitefly vectors. Maize streak virus is a representative of the first type<sup>15</sup>; the vector of cassava latent virus is not known. However, bean golden yellow mosaic virus has a whitefly vector, and also contains single-stranded DNA, of estimated molecular weight  $0.66-0.95 \times 10^6$  (refs 8 and 16). We see in this and other similarities the emergence of a new virus group, comprising the plant viruses with small paired particles. The name geminivirus is proposed for this group, which will be described in detail elsewhere (unpublished data of K. R. B.).

Our work has provided no support for the suggestion<sup>7</sup> that the genome of maize streak and cassava latent viruses is in two parts contained respectively in the two particles that make up a pair. If, as we suggest, one of the nucleic acid species separated by gel electrophoresis is derived from the other, the DNA genomes would seem the smallest known for independently replicating viruses.

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1. Bock, K. R., Guthrie, E. J. & Woods, R. D. *Ann. appl. Biol.* 77, 289-296 (1974).
2. Mumford, D. L. *Phytopathology* 64, 136-139 (1974).
3. Matyis, J. C., Silva, D. M., Oliveira, A. R. & Costa, A. S. *Summa Phytopathologica* 1, 267-274 (1975).
4. Galvez, G. E. & Castano, M. *Turrialba* 26, 205-207 (1976).
5. Goodman, R. M., Bird, J. & Thongmearkom, P. *Phytopathology* 67, 37-42 (1977).
6. Bock, K. R. & Guthrie, E. J. in *African Cassava Mosaic* (ed. Nestel, B. L.) 11-16 (International Development Research Centre, Ottawa, 1976).
7. Bock, K. R., Guthrie, E. J., Meredith, G. & Barker, H. *Ann. appl. Biol.* 85, 305-308 (1977).
8. Goodman, R. M. *Nature* 266, 54-55 (1977).
9. Rose, J. A., Berns, K. I., Hoggan, M. D. & Koczot, F. J. *Proc. natn. Acad. Sci. U.S.A.* 64, 863-869 (1969).
10. Espejo, R. T., Canelo, E. S. & Sinsheimer, R. L. *Proc. natn. Acad. Sci. U.S.A.* 63, 1164-1168 (1969).
11. Zuccarelli, A. J., Benbow, R. M. & Sinsheimer, R. L. *Proc. natn. Acad. Sci. U.S.A.* 69, 1905-1910 (1972).
12. Davis, R. W., Simon, M. & Davidson, N. *Methods in Enzymology* (eds Grossman, L. & Moldave, K.) 21D, 413-428 (Academic Press, New York and London, 1971).
13. Sinsheimer, R. L. *J. mol. Biol.* 1, 43-53 (1959).
14. Dingman, C. W., Fisher, M. P. & Kakefuda, T. *Biochemistry* 11, 1242-1250 (1972).
15. Storey, H. H. *Ann. appl. Biol.* 12, 422-439 (1925).
16. Goodman, R. M. *Virology* 83, 171-179 (1977).
17. Murrant, A. F., Mayo, M. A., Harrison, B. D. & Goold, R. A. *J. gen. Virol.* 16, 327-338 (1972).
18. Biddlecombe, W. H. *et al. J. Physiol.* (in the press).

# reviews

## Animal signal characteristics

P. J. B. Slater

*Optical Signals: Animal Communication and Light.* By Jack P. Hailman. Pp. xix + 362 (Indiana University: Bloomington, Indiana and London, 1977.) £11.25. *Animal Communication.* Second edition. By Hubert and Mable Frings. Pp. ix + 207. (University of Oklahoma: Norman, Oklahoma, 1977.) Hardback \$9.95; paperback \$4.95.

THE striking and often stereotyped displays with which animals communicate have always fascinated ethologists. The similarity of signals within a species, and the differences between species, make them well suited to the study of behavioural evolution. By examining the contexts in which signals occur, some progress has also been made towards understanding the motivational systems underlying them and thus the messages which they convey. On the other hand, the selective forces which have led them to be as they are have received less attention.

It is on this last area, the ways in which signals are adapted to the environments in which they occur and to the functions which they have, that Hailman hopes to shed some light. He was originally asked to write a chapter on visual signals for a book to be edited by Sebeok but, having over-run his word limit, he decided to use the additional material for a book of his own. His aim in this is a limited one: "to specify optical principles, search for ecologically relevant situations, and then predict animal signal characteristics". As this is a largely unexplored area, the range of topics covered is unconventional for a book on animal communication.

The first two chapters outline the author's approach to the problem, dealing in particular with scientific philosophy and information theory. The following three are concerned with the properties of the channel, the sender and the receiver: in effect, optics, the basis of animal colours and movements, and visual sensation and perception. These chapters provide a wealth of background material covering every physical and physiological process which might conceivably be relevant to the form of animal signals. The rest of the book gets down to examining the sorts of signals which

should emerge, given these constraints. A chapter on deception, dealing largely with crypsis and mimicry, is followed by one on noise, in which the ways in which animals may achieve conspicuousness in various situations are explored. A further chapter deals with the sorts of signals which might carry different types of information.

The book is a bold attempt to take a novel approach to visual communication, but in many ways it is hindered by the very novelty of that approach. Although the background information in the earlier chapters is solid and well-established, the later ones sink into the haze which often surrounds functional questions. To approach such problems as "Why is the cardinal red?" one can only resort to correlational methods with all their attendant difficulties, looking across species to see if red colouration occurs in some situations more than in others. The correlations the author uses are neither extensive nor formal, so that many of his suggested explanations rest on speculations from selected data. He has, however, done a service by pointing to an inter-

esting and neglected area. If he has been unable to pull all the strings together himself, he has at least provided a basis on which research towards doing so may be founded.

Hailman's book is tough reading: it is tersely written and contains few detailed examples; the author has a liking for classifying things with the aid of a confusing variety of polysyllabic labels; the text is in typescript form and contains many misprints. Although also poorly produced (two pages are even printed in the wrong order), the book by Frings and Frings is something of a contrast, as it was written as a brief introduction for laymen, and contains many examples and little theory. Such theory as it does include is, however, seriously out of date, for it was originally published in 1964 and the new edition has been simply updated by adding a chapter on recent advances. This is an unhappy compromise which can hardly do justice to what has happened since then. □

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## Lunar studies

*The Moon: A New Appraisal from Space Missions and Laboratory Analyses.* Edited by G. M. Brown, G. Eglinton, S. K. Runcorn and H. C. Urey. Pp. vi + 606 + 15 plates. (Royal Society: London, 1977.) £38 in the UK; £38.95 overseas.

THIS book is a collection of papers given at a Royal Society symposium in 1975. Many, perhaps most, of the leading research groups in lunar science are represented. It represents well the state of the subject at the time of presentation.

The climactic period of lunar studies began with the Apollo missions in 1969–72, but it did not end there. The rich collection of lunar samples returned by US and Soviet missions, the experiments left on the moon, and the data tapes obtained during the missions, have provided the basis for a vigorous and even an expanding science. Although the work has been published in all the appropriate journals (including *Nature*), it has a special point of concentration in the Proceedings of the Lunar Science Conferences held each year in Houston. The worker seeking direct access to some

aspect of lunar research should begin there.

What then is the role of the present volume? The editors have made a deliberate, and quite successful, effort to make the work accessible to a wider scientific public. Authors have been encouraged to begin at the beginning, and to set their work in context. As a result, some of the papers are admirable introductions to important and difficult subjects. Others are broad-brush extended abstracts, which can at least alert the reader to the significance of an area, or the point of view of one group. Narrower, more technical papers have not been eliminated, but they do not dominate the whole.

The first section, on the accumulation and bulk composition of the moon, is especially good. The evidence, mainly isotopic and chemical, is presented with care by Wasserburg, Anders, Wänke, and Lal *et al.* I know of no more accessible current summaries of the beautiful development of early lunar chronology, or of the striking patterns of trace element abundances.



The organisation of such a complex subject is always a problem. Certain articles in later sections are very close to this group, and should in my judgment be read with them. A later article by Palme and Wänke, one on lunar gravity by Sjogren, and three in the last section beginning with that of Kopal, are among these. They bear closely on questions of origin and early history.

The period of mare formation, extending roughly from  $4 \times 10^9$  to  $3 \times 10^9$  y ago, was an active stage about which we know a great deal. The least clear point is the relationship, causal and temporal, between the major impacts which produced huge basins on both the front and back sides of the planet, and the basaltic flows which created the maria on the side we see. This is a major theme of Section III; the articles by Geiss *et al.* and by O'Hara *et al.* deal especially with important aspects of the subject.

The meteoritic bombardment of the lunar surface continues to the present. It is important in its own right, and also as a chronological tool. A direct comparison of ages in different lunar regions is possible, and to some extent the chronology can be given an absolute basis, using the lunar samples. The comparison with other planets, especially Mercury and Mars, is more difficult. Articles in this section by Neukum and Housley, among others, are notable. Signer *et al.* call attention to some gaps in our understanding of lunar rare gases. Strangway and Olhoeft, and others, demonstrate the importance of electrical

measurements in understanding the interior. Coleman and Russell, Runcorn, and others, summarise our knowledge of the striking magnetic remanence seen in the lunar samples, and in the orbital data. The coverage of geophysical aspects of lunar science is in fact especially thorough.

Allowing for the fact that any symposium must contain some weak contributions along with the strong, this volume can be highly recommended. Its one general fault is shared with many others: it appears more than two years after the event. There have been remarkable developments since, which cannot be reflected here and must be found in the current literature. Chronological and isotopic studies, comparative lunar and meteoritic work, and the interpretation of orbital remote sensing data are among the areas that have moved ahead rapidly since 1975.

The distinguished editors have chosen to close the volume with an "Epilogue" showing a colour plate of Apollo orbital geochemical data, and pointing out the importance, for our understanding of the origin of the moon and the Solar System, of a global survey by a complete set of geochemical and geophysical instruments. This describes a potential future mission, the Lunar Polar Orbiter. Since this is a cause to which this reviewer has been passionately committed, he can hardly fail to agree with these sentiments.

James R. Arnold

James R. Arnold is Professor of Chemistry at the University of California at San Diego.

## Charged particle beams

*The Physics of Charged-Particle Beams.* By J. D. Lawson. Pp. xxi + 462. (Oxford University. (Clarendon): Oxford; 1977.) £16.50.

CHARGED PARTICLE BEAMS have served to probe the nucleus, to analyse minute quantities of material, to reproduce television images, to resolve living structures smaller than the wavelength of light, to cut and join metals, and to reveal the subnuclear universe, as tiny and unexpected as that of the astronomer is large and apparent. In the future, one hopes they will provide the means to contain and perhaps ignite fusion reactions and prove useful in the treatment of malignant disease.

The field is as diverse in its intellectual content as in its applications. Based firmly in classical electromagnetism, it extends to a mathematics of collective phenomena as fascinating as any to be found in the fields of pure science to which it has been applied.

Lawson is particularly well placed, having contributed to most branches of this field of applied physics, to write an authoritative and unifying text. This he does, manfully striding through six chapters of somewhat daunting mathematical equations, from some which the under-graduate will recognise to others which still puzzle the expert.

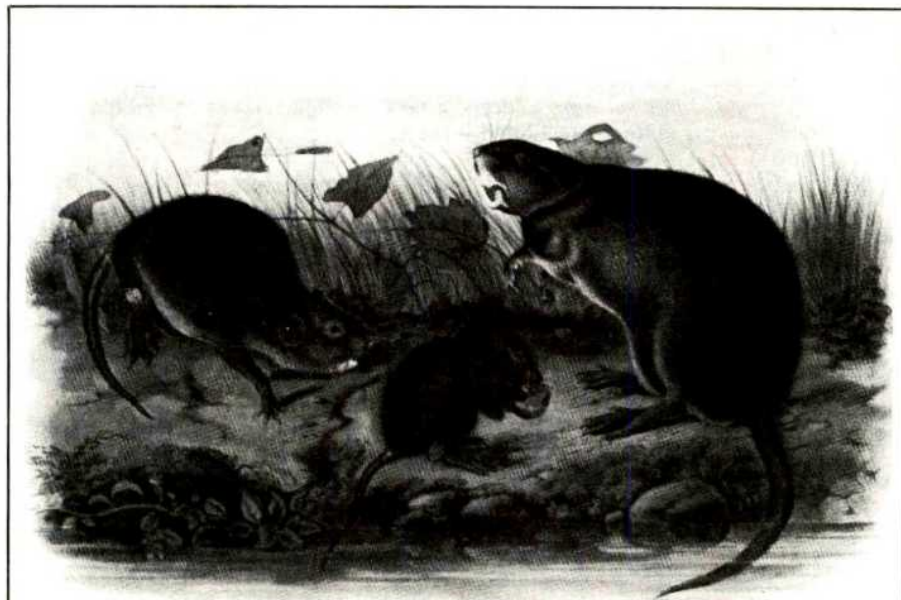
Besides the achievements of charged particle beam physics, the book stands as might a great master's treatise on perspective against the riches of renaissance art, perhaps somewhat disappointing to those who would appreciate but invaluable to those who would contribute.

The material is organised in an intellectual sequence which will frustrate those who may seek mathematical assistance in the solution of a particular problem. But no matter, it should be read from cover to cover, for Lawson disregards the partitions between specialities in the field in an attempt to cross pollinate neighbouring branches of the tree of physics whose blossoms, beautiful as they may be, might otherwise begin to show signs of infertility.

Such a book has been long awaited, particularly by those of us in the particle accelerator and fusion fields. It is regrettable that its printers have reverted to a typescript presentation that would have offended Gutenberg. Provided the reader can overcome his aesthetic scruples, however, he will find the layout of equations, figures, references and indexing perfectly adequate.

E. J. N. Wilson

E. J. N. Wilson is a senior physicist in the team which constructed the 400 GeV Super Proton Synchrotron at the European Organisation for Nuclear Research (CERN), Geneva.



Family of muskrats (*Ondatra zibethica*). By John James Audubon in his famous book *Quadrupeds of North America* (1849). Illustration taken from *Larousse Animal Portraits*, compiled by P. P. Grasse (Hamlyn: London, New York, Sidney and Toronto, 1977; £6.95). The book contains over eighty Natural History prints from the seventeenth to the twentieth century, a large proportion of which were selected from a collection in the Natural History Museum in Paris. The collection was started by a brother of Louis XIII, Gaston of Orleans, a keen naturalist and patron of the arts.



## Experimental design and statistical analysis

*Experiments: Design and Analysis.* Second edition. By J. A. John and M. H. Quenouille. Pp. 296. (Charles Griffin: London and High Wycombe, 1977.) £12.

*The Design and Analysis of Experiment* by Quenouille, first published in 1953, was an early text which emphasised the practical aspects of experimental design and statistical analysis. The second edition, which appears under the joint authorship of J. A. John and Quenouille, who died at an early stage in its preparation, preserves the spirit of the original, with detailed coverage of the more widely used designs fully illustrated by numerical examples from either agricultural or animal experiments.

Despite the high standard of the original, the changes incorporated in the new edition are welcome; the grouping of chapters into sections has been abandoned and some re-arrangement has allowed the inclusion of two new chapters—one on fractional replication and the other on response surface methods—without increasing their total number. The contents have been updated with advances made since first publication, and strengthened with further elaboration of specific designs. The bibliography has been revised and expanded.

The first four chapters discuss randomised blocks, Latin squares, and factorial and split-plot designs. Techniques for missing-values, originally confined to a separate chapter, have been inserted at the appropriate places. Discussion of missing-values in randomised blocks is particularly extensive, although it is strange that two missing-values in such designs should be estimated by iteration rather than directly equating the appropriate residuals to zero.

The next four chapters illustrate the main modifications of full factorials through confounding and partial replication. The new chapter 9, on response surface methods, provides an excellent account of first- and second-order designs, including rotatability; examination of the fitted response surface is particularly well illustrated.

Chapter 10, on incomplete block designs, includes recent work on resolvable and cyclic designs and a brief introduction to partially-balanced incomplete blocks. The three chapters (11, 12 and 13) on designs for estimating residual effects in long-term experiments, planning of groups of ex-

periments and combination of results, still constitute one of the few adequate expositions of these important topics. The final chapter describes the scaling of data, and the book ends with tables of  $t$ ,  $F$ ,  $\chi^2$ , studentised range and random permutations, a bibliography and a good index.

This book is a valuable guide to the understanding of experimental design: its discussion of real data and the

absence of mathematical and statistical models should appeal to the experimenter. Its comprehensive coverage of the simpler designs and the tabular summaries of their properties provide a useful reference for the statistician.

A. L. Johnson

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## Population differentiation and gene-flow

*Geographic Variation, Speciation, and Clines.* By John A. Endler. Pp. ix+246. (Princeton University: Princeton, New Jersey, 1977.) Hardback £12; paperback £5.45.

CHARLES DARWIN, with his knack for picking a good problem, wrote: "Those forms which possess in some considerable degree the character of species, but . . . are so closely linked . . . by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us." Endler's book reviews the diverse and scattered literature on clines—that is, the study of population differentiation in the absence of barriers to gene-flow—that has accumulated since Darwin.

It directs itself to two fundamental questions. First, it asks whether sharp geographical differentiation can evolve across a spatially or genetically continuous series of populations; or whether gene-flow can prevent spatial differentiation. The answer is rather clear. Subspecific and even specific differentiation (parapatric speciation) in the absence of barriers to gene-flow is as common and important as divergence with strong barriers, whatever our introductory textbooks might say.

Second, it asks about the shape of clines: for example, whether stepped clines require stepped environments and whether there is always a unique one-to-one correspondence between local steepening and environmental change. Endler explores the rich possibilities in considerable detail, using analytical and computer models, as well as laboratory populations. The fairest summary is that "it depends". Chapter 4, in particular, shows how conclusions based on classical single-locus models can be much influenced by the effects of co-adapted modifiers.

Whereas chapters 2–4 consist of rather detailed population genetics, the last two chapters, which they underpin, are of more general interest. Chapter 5 elaborates the earlier con-

clusion that parapatric speciation is probably common. Indeed, present-day patterns of distribution resulting from parapatric speciation do not differ significantly from those generated by allopatric speciation and secondary contact. Even the familiar explanations for present-day distribution patterns of Amazonian and African forest birds, butterflies and other organisms, based on Pleistocene refugia within forest remnants and subsequent secondary contact, look shaky in the light of Endler's reanalysis in chapter 6. These distributions can equally well be explained by the presence of some kind of selection gradient, which may be sufficient to maintain geographical differentiation regardless of whether or not the forests have been fragmented.

The book summarises a great deal of empirical data on clines of all kinds, and makes a brave attempt to match theory with data wherever possible. Efforts to assign numbers to key parameters—for example, rates and distances of gene-flow and cline width (the distance over which gene-frequencies change)—are particularly commendable. Many of the estimates may be no better than an order of magnitude, but they can nevertheless constrain the biologically feasible solutions to general models in a dramatic and cautionary way. Nevertheless, I was left with the distinct impression that theory can now generate so many alternative explanations for apparently simple phenomena that distinguishing hypotheses would in many cases be a major undertaking, if not downright impossible.

Before I read the book, I knew very little about clines, other than that they were interesting. When I was reading it, there were times when I felt that I was finding out more than I really wanted to know; that somehow, Darwin's clear, simple problem had been lost in the toolkit of modern population genetics. But by the time I finished it, I was more convinced than ever that clines are intriguing. For any biologist brought up in the simple belief that allopatric speciation is all, this book is essential reading.

John H. Lawton

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## African fossil hominids

*Catalogue of Fossil Hominids. Part 1: Africa. (Second edition). Edited by K. P. Oakley, B. G. Campbell and T. I. Molleson. Pp. 210 + 21 plates. (British Museum Natural History: London 1977.) £12.*

REVIEWING a catalogue can be likened to reviewing a telephone directory. It would take a devoted reviewer—nay, an obsessional one—to check all the facts; all one can reasonably do is comment on their presentation.

There are now three published parts of the *Catalogue of Fossil Hominids*. Part I deals with Africa, Part II with Europe, and Part III with the Americas, Asia and Australasia. Part I is the first to go into a second edition. All follow a similar style. Fossils are grouped by site and then, for the large sites, into anatomical subcategories. Eighteen items of information are given about each fossil, or group of fossils, varying from the exact name and location of the site to the location of the moulds for casts. The items include a very brief description of the completeness of the fossils, as well as geological, palaeontological and bibliographic information. The *Catalogue* is clearly intended as a source for reference and information about hominid fossils; how well does the series, and this particular volume, fulfil this role?

As a comprehensive source of information, the series has no rival. My experience is that the volumes represent a very reliable source of data. I detected remarkably few errors in this volume, and the prodigious task of checking entries and proof-reading has been carried out with considerable skill. Once one is accustomed to the layout, information is comparatively easy to retrieve. The use of a bookmark as a key to the various items of information is an excellent idea. (Publishers of Tolstoy, Pasternak and Trollope, please note!)

I would have welcomed clearer information about the circumstances of many of the fossil finds. Were they found 'in situ' or on the surface; and, if on the surface, how reliably can they be associated with particular horizons? Taxonomic attributions are given, but they tend to be hidden away between citations of original reports; perhaps they could be given a section of their own? Inevitably, the choice of which papers to cite is subjective, but I would quibble with some of the selections. It is unfortunate that the paper cited as the first report of KNM-ER 1470 does not mention the specimen in the text at all.

The inclusion of site maps is a welcome introduction, and greater use of geological sections should be encouraged, though it has to be admitted that compound sections can appear misleadingly simple. The decision to continue the use of Nomina Anatomica terms to describe the material is a wise one: even if it does lead to 'ossa facie'

and 'face' being used in the same sentence, it is important to base descriptions on internationally understood terms. The inclusion of photographs of so few specimens, even if they are holotypes, is of questionable value.

I had to work very hard to find even minor points of criticism in the latest volume. Fossils from Sterkfontein are catalogued within Members, but none of the references cited gives information about the definition of the Members; and in the section on Swartkrans reference is made to A4 dating, but this category of dating is not defined in the general introduction; however, these are points of detail and not serious criticisms.

There is no doubt in my mind that the quality of the whole *Catalogue*, and this new volume in particular, is a credit to the editors, and to the scientists who contri-

buted entries from individual countries. The Wenner-Gren Foundation for Anthropological Research is to be congratulated for supporting the preparation of the *Catalogue*. The clear layout and high quality of the presentation do credit to the publishers, who are to be commended for producing such a specialist publication at a realistic price. The *Catalogue* will continue to accumulate entries, and one hopes that the possibility of producing it in a loose leaf format is being explored.

My own copy of the predecessor to this new edition is so well used that it is disintegrating. I am confident the same fate will befall the new edition; I can think of no finer recommendation for a book.

**Bernard Wood**

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## Statistical techniques in biostratigraphy

*Concepts and Methods of Biostratigraphy. Edited by Erle G. Kauffman and Joseph E. Hazel. Pp. xiii + 658. (Wiley: New York, London and Toronto, 1977.) £26.25; \$44.45.*

So much in geology depends on good biostratigraphy that one must welcome any good new treatment of its concepts, methods and results. This weighty (in more ways than one) volume starts well with a scholarly and absorbing essay by J. M. Hancock on the history of concepts of correlation, and continues with six articles on a variety of biological concepts involving, for example, speciation, dispersal mechanisms and biogeography. There follow a further six articles on biostratigraphic methods, and the remainder of the book deals with a dozen major fossil groups. These include such excellent stratigraphic indices as graptolites, conodonts and ammonites together with a few 'jokers', such as corals and gastropods, which no respectable stratigrapher would use if he could possibly avoid it. Even more surprisingly, there is nothing on the increasingly important nannofossils and dinoflagellates, that have in recent years become extremely valuable in much oil company exploration and deep-sea drilling.

Although the book is marred somewhat by the poor, smudgy quality of many of the illustrations, the editors are to be congratulated on having persuaded so many leading palaeontologists to collaborate in producing a volume that will serve as a most useful teaching aid and reference work. If I

find myself more than a little resistant to the promotional enthusiasm of the blurb writer, it may be because I am not persuaded by what I estimate to be the editors' higher ambitions, judging from their joint preface and individual articles. Hazel wishes to promote the use of a variety of statistical techniques in biostratigraphy, but in my experience good stratigraphic marker fossils are the only crucial consideration. If they are present then statistics are unnecessary and if they are not, no amount of juggling with numbers will be of much help.

Far more interesting in my view is Kauffman's ambition to apply modern evolutionary and ecological concepts to improving correlation as exemplified by his work on the Cretaceous of the US Western Interior. Although I share his fascination with the sorts of biological and geological questions that his work raises, I feel he may perhaps be guilty of putting the cart before the horse. Biostratigraphy is essentially an empirical subject and excellent work can be undertaken by people with little interest in, knowledge of, or talent for biological theory (one has only to think of stratigraphy's prime exemplar, William Smith). Thus, ammonites are more useful in stratigraphy than bivalves because of their much higher rate of faunal turnover, a fact recognised and utilised for well over a century. This raises an intriguing scientific question, as does, says, the existence of faunal provinces, but in both cases attempted solution of the problems is irrelevant to correlation of strata. Good palaeobiology depends on good stratigraphy and not the other way around.

**A. Hallam**

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# obituary

## A. R. Luria

ALEXANDER ROMANOVITCH LURIA, the distinguished Soviet neuropsychologist, died in Moscow on 14 August, 1977, at the age of 75.

He was born in 1902 and educated at the Universities of Kazan and Moscow, where he took his degree in psychology, later qualifying also in medicine. A talented experimental psychologist, his best known work was concerned with the analysis of psychological disabilities resulting from war wounds of the brain. But his interests covered a wide range of themes in experimental psychology, in particular problems of language and its acquisition in young children. He paid several visits to this country and a number of his books have been translated into English.

Luria's earliest work was concerned with the analysis of motor disorganisation provoked by conflict and stress. This was based on a long series of experimental studies carried out at the State Institute of Experimental Psychology in Moscow in the 'twenties and published as a book which appeared in English translation in 1932. Although deriving in some measure from Jung and Peterson's work on word-association and the psychogalvanic response, Luria was concerned with the disruption of volitional movement rather than with autonomic changes and did much to extend the use of objective method in the study of emotional stress.

Rather surprisingly, his work owed but little to Pavlov, who at much the same time was investigating experimental neuroses in dogs by conditioned reflex techniques. While Luria held Pavlov's physiological work in the highest esteem, relations between physiology and psychology in the Soviet Union have never been close and few Soviet psychologists espoused behaviourism. As with the great majority of Soviet psychologists, Luria's outlook was far from Pavlovian and his work was wholly confined to the study of human experience and behaviour.

The most important figure in Russian psychology after the Revolution was L. S. Vygotsky, Luria's predecessor in the Chair of Psychology in the University of Moscow and a man who influenced him greatly. Vygotsky's main interests lay in child development, more especially in the study of



language and its relation to intellectual growth. Following Pavlov's suggestion that the main differences between human and animal behaviour are bound up with the evolution of language, Luria concerned himself particularly with the acquisition of language in young children and its effects on the learning of motor skills.

Although relatively little of this work was translated, he brought much of it together in a series of three lectures delivered at University College London in 1958 and subsequently published as a short book under the title of *Speech and the Regulation of Normal and Abnormal Behaviour* (1961). More physiological in tone than Vygotsky, Luria followed him in his full acceptance of the importance of language in the intellectual development of the child. In this respect he stands far closer to Piaget than to Pavlov.

Interested as he was in the acquisition of language, Luria was even more concerned with the nature of its dissolution. His studies of aphasia, somewhat in the tradition of Hughlings Jackson and Henry Head, remain perhaps as his outstanding contribution to neuropsychology. During the last war, Luria was closely concerned with the assessment of psychological disability in brain-injured servicemen and in formulating programmes of rehabilitation based upon thorough psychological examination. His book on *Traumatic Aphasia* (1947, English translation 1970), is especially noteworthy for its detailed analysis of the various patterns of linguistic disability resulting from brain injury and their

relationship to the site and extent of the cerebral lesion.

Some years later, his considered views on the problems of cerebral localisation together with an exposition of his methods for eliciting specific psychological deficits were set out in a noteworthy monograph. This was *Higher Cortical Functions in Man* (1963, English translation 1966), which has had wide influence. Although putting forward no new theory or model of higher nervous activity, the subtlety and intuitive skill displayed by Luria in devising simple methods for analysing cortical defects and evaluating their significance represents a major achievement.

Apart from his scientific preoccupations, Luria was a man with lively human sympathies who achieved an unusual degree of empathy with the victims of brain injury. Among these was the distinguished physicist, L. D. Landau, to whose rehabilitation after a severe head injury Luria devoted quite outstanding skill and care. Nor were his ministrations limited to the famous, as is witnessed by his moving account of the visual and symbolic difficulties of a completely unknown young scientist who had sustained a penetrating occipitoparietal war wound of the dominant cerebral hemisphere. This case is described in *The Man with a Shattered World* (1972), a semi-popular book written with unusual sensitivity and very considerable literary skill. As has been well said elsewhere, Luria was a very warm human being and a deeply compassionate and concerned physician with something of the artist in his composition.

Alexander Luria was a holder of the Order of Lenin and received many other honours from his own country and further afield. He was a foreign member of the National Academy of Sciences of the United States and an Honorary Fellow of the British Psychological Society. He was awarded an Honorary Doctorate of Science by the University of Leicester. He is survived by his widow, herself a medical scientist, and daughter.

O. L. Zangwill

## Jean Maetz

JEAN MAETZ died tragically in a road accident in Scotland on 16 August, 1977. He was Chief of the Groupe de Biologie Marine of the Département de



Biologie du Commissariat à L'Energie Atomique located at the Station Zoologique, Villefranche-sur-Mer, France. Born in Mutzig in Alsace in 1922, he was educated at the Ecole Normale Supérieure in Paris, and worked at Saclay before going to Villefranche in 1964.

Early attempts to measure teleostean osmoregulation with radioisotopes had been made in the fifties, and revived by Motais in 1961. Maetz with Motais in 1965 discovered that in sea-water the gill was the major site of sodium exchange, and that up to 100% of the internal sodium could be exchanged each hour. Maetz was quick to realise the importance of techniques which allowed both influx and efflux of ions across the gill to be measured while the salt balance of the animal was not disturbed. He refined and automated these techniques with the effect that a constant stream of papers has flowed from his laboratory in which a variety of perturbations (ionic strength, temperature, hormones) were employed and their effects on branchial fluxes described. He was particularly interested in problems of adaptation in euryhaline fishes and while he stressed the difficulty of obtaining exact thermodynamic data in whole animals he enjoyed building models to describe the data. These were intended as a challenge to others to do experiments designed to test the models.

It is in no small part due to his stimulus that there is a lively interest in branchial transport today. While there is now general agreement about the active transport of chloride in the seawater gill, the mechanism of Na-K exchange is more controversial. Whether exchange proceeds by an active mechanism or is a result of potential changes accompanying alterations in composition of external medium is still a matter of dispute. Although Maetz with Campanini in 1966 were the first to show the change in gill potential which followed when eels were transferred from sea water to fresh water Maetz initially favoured an active mechanism. More recent observations suggest that Na/K exchanges are diffusive in nature, yet awkward observations remain to indicate that some component of the exchange is potential insensitive. It is a tragedy he will not be able to resolve this matter after contributing so much of the information on which the discussion is based.

The impact of the mass of phenomenological data collected for many different species, which has come from his efforts has perhaps not yet been realised. Its importance to ecology and relevance with regard to the changing environment of the seas and estuaries may be considerable.

More recently he turned to simpler systems such as the perfused gill, work already started with Rankin, and to biochemical and morphological studies of chloride cells. It is to be hoped others will continue to explore his ideas in these areas.

Jean Maetz was a man of great charm with an infectious enthusiasm for research. Many of his former students now hold positions in research institutions and his laboratory was never without visiting scientists, who came from all over the world. A visit to his laboratory was a commitment to do experiments, and often at the end of the day to enjoy the hospitality and friendship offered by his whole family. He is survived by his wife, Betty, and two daughters. *A. W. Cuthbert*

## Louis Fieser

LOUIS FREDERICK FIESER was an exemplar of excellence promoted and sustained by Harvard University. Born in Columbus, Ohio, on April 7, 1899, he completed his Ph.D. degree under J. B. Conant at Harvard in 1924. After five years on the staff of Bryn Mawr College (where he discovered his aptitude for teaching) he returned to the Harvard chemistry department in 1930, becoming Sheldon Emery Professor of Organic Chemistry (1939–1968) and Professor Emeritus from 1968 until his death on July 25, 1977.

Among the honours conferred on Fieser were Fellowship of the National Academy of Sciences (1940), and the D.Pharm. *honoris causa* of the University of Paris (1953). From the American Chemical Society he received the Wm. H. Nichols Medal (1963) and the Award in Chemical Education (1967).

Fieser's abounding enthusiasm for investigative organic chemistry was rooted in his own experimental work, which he continued throughout his career. His attachment to bench work is well portrayed in the photograph that accompanied a biographical sketch by Dr Hans Heymann in the *Journal of Organic Chemistry* of June 1965—an issue composed of numerous papers dedicated to Fieser on the occasion of his 66th birthday, by authors from many countries who had been students or post-doctoral workers in the Converse Memorial Laboratory.

Early research by Fieser stimulated developments in several fields which have remained important. In 1939, he established the structure of Vitamin K<sub>1</sub> by a synthesis of characteristic practicality, isolating the natural vitamin from alfalfa extract for comparison. Fieser had earlier devised syntheses of methylcholanthrene and other carcinogenic hydrocarbons, and his varied interests led him to compile (with Mary

Fieser) his books on *Natural Products Related To Phenanthrene* (1936, 1937) in which the developing (and confused) chemistry of the steroids was skilfully elucidated and critically reviewed. In the 1937 volume, Fieser introduced the  $\alpha/\beta$  nomenclature now universally employed to designate hydroxy and other substituent configurations in the steroid nucleus. The 3rd edition appeared in 1949, benefiting from (and contributing to) the burgeoning of steroid research that accompanied the discovery of the therapeutic value of cortisone.

The ensuing decade saw remarkable advances in steroid chemistry and biochemistry which had an impact in much wider fields. Observations by Fieser on the stereochemistry of steroid reactions were among the stimuli that led D. H. R. Barton (while a Visiting Lecturer at Harvard) to develop the principles of conformational analysis. In addition to participating in many aspects of research on steroids, Fieser found time to compile—again with his wife as co-author—the masterly 4th edition of the original book, necessarily retitled *Steroids* (1959) because the wealth of new information compelled the exclusion of other material.

Fieser's insight in selecting significant research topics is exemplified by his early work on benzo[*a*]pyrene, the carcinogenic action of which has only recently been clarified in structural terms. Another of his interests concerned the possibility that carcinogenicity might arise from minor sterols occurring as companions of cholesterol: he isolated a number of such minor components from commercial cholesterol. Although no carcinogenic sterols (with the exception of cholesterol  $\alpha$ -oxide) appear to be known, recent work has indicated that certain compounds such as 25-hydroxycholesterol may have significant biochemical effects.

Fieser himself considered that success in teaching was his major accomplishment. At Harvard, his attractive informality as a lecturer won him the students' acceptance of a rigorous introductory course in organic chemistry, generally regarded as 'tough, but well worth it' by the participants—who numbered more than 8,000 during the 30 years of the course. Experimental skill was assiduously inculcated, as in the annual Martius Yellow prize competition for the practical class, in which 'Louie' frequently took part.

Fieser's earliest textbook, *Experiments in Organic Chemistry* (1935), and its painstakingly revised successors, conveyed up-to-date techniques to students world-wide: the colour pictures showing how to construct a hot-plate from a can labelled 'Gorton's Cod Cakes' impressed even those readers

who were unacquainted with those comestibles.

The 1957 edition of *Experiments* included an informative review of some 320 reagents: from this modest chapter, Mary and Louis Fieser were to develop their monumental series of handbooks entitled *Reagents for Organic Synthesis*—five volumes comprising more than 4,000 pages of invaluable information and references for research workers.

During the Second World War, Fieser's attention was diverted to investigations on antimalarial drugs of naphthoquinone type. He also played a leading role in the development of incendiary preparations, including napalm. It is clear from his personal account of this work, in the book *The Scientific Method* that while Fieser relished this challenge to the skill of his research team, he was impatient to resume his own research at the earliest opportunity.

Meanwhile, his enforced travels on war service provided some spare hours in which he began (in collaboration with Mary) the first of a series of sparkling and scholarly textbooks on organic chemistry, in which the excitement of discovery was conveyed in a lively, lucid and gracefully literate style. These works deservedly achieved great international success, both in the original and in translations. The first Japanese edition of the *Textbook of Organic Chemistry* appeared in 1952 just as peace was formally resumed between the U.S.A. and Japan: its frontispiece portrayed the Fiesers' Siamese cat accompanied by one of Japanese breed, symbolising the authors' hope that their work would promote reconciliation.

In later years, the Fiesers produced two further books for advanced students, *Advanced Organic Chemistry* (1961) and *Topics in Organic Chemistry* (1963) together with a *Style Guide for Chemists* and other works. Fieser also promoted the study of stereochemistry by means of his inexpensive molecular models based on those of Dreiding.

It was typical of Fieser's determination that after undergoing surgery for a lung tumour he became a campaigner on the risks of smoking, in addition to pursuing his customary literary work. The elegance of the latter matched that of his Siamese cats, but in his perseverance Fieser was more appropriately described by his mottoes, which were *Omnia possum* and *Labor omnia vincit*.

The human warmth implicit in Fieser's books prompted affection for the author in many readers who had no opportunity of direct contact with him. Friends and disciples known and unknown will salute the memory of a

creative and courageous chemist, and offer their condolences to Mary, his wife and scientific partner throughout his career.

C. J. W. Brooks

## Sir Frederic Williams

PROFESSOR Sir Frederic Williams, FRS, who died after a year-long illness on 11 August 1977 had held the Edward Stocks Massey Chair of Electro-Technics at the University of Manchester since 1946; he was famed for his pioneer work on electronic circuitry and digital computers, and was perhaps less well known for his variable-speed induction motors and his new transmission system for a motor-car.

Frederic Calland Williams was born on 26 June 1911. He was educated at Stockport Grammar School, the University of Manchester (B.Sc. and Fairbairn Prize 1932, M.Sc. 1933, D.Sc. 1939) and Magdalen College, Oxford (D.Phil. 1936). He joined the staff of the Electro-Technics Department at Manchester in 1936, but left in 1939 to join the Scientific Civil Service at Bawdsey. During the war he worked on radar at TRE (Telecommunications Research Establishment; now the Royal Radar Establishment). It was at TRE that his inherent ability in circuitry became apparent. Those were the days of valves and he used them in unconventional ways to generate the waveforms required in the rapidly developing field of radar. There were circuits actively using all the electrodes of a pentode; diodes were employed for 'catching'; feedback (both positive and negative) was used. It is believed that Williams introduced the 'virtual earth' idea which so simplifies the analysis of negative feedback amplifiers. At TRE he also made contributions to servo-mechanisms.

When he became Professor at Manchester, all the war-time experience was incorporated in new courses which he gave to undergraduates (the whole staff attending, too). This experience also enabled him to start new research lines in his department. Most significantly, he brought to Manchester an idea for a digit store for a computer. There were computers in 1946, but they lacked storage. The Williams device stored binary digits as a charge pattern on the screen of a cathode ray tube. The pattern was scanned and read and continuously 'refreshed' by re-writing from the read-out. It was possible to inspect any point of the pattern at random; access time was thus shorter than in acoustic delay lines—a rival system of storage being developed elsewhere.

A complete prototype computing machine incorporating the Williams tube store was built in the laboratory

at Manchester and a commercial version was produced by Ferranti Ltd., who installed about 20 computers in various establishments in this country and abroad. Early in the development of the second Manchester machine, Professor Williams' interest in computers waned and he took up other interests. However, he must surely be of that small company of men deserving the title of 'Father of the Computer.'

The new interest was induction machinery. The induction motor is essentially a constant-speed machine, although there are machines with two separate windings either of which can be excited to give a two-speed machine. Williams sought to make an induction motor whose speed could be continuously variable over a range and all of whose windings were used all the time. There was the disc motor derived from the linear induction motor; this was a flea-power machine which demonstrated the practicability of the idea, but it led to the spherical motor in which almost the whole surface of the rotor was in active use. A large motor of this type was made by Metropolitan-Vickers Electrical Company, but the machining of spherical surfaces proved difficult and this may be why no further machines were made.

There followed the 'log-motor' (a machine of amazing ingenuity), the phase-change motor (in which two-thirds of the windings were supplied through variable phase changers), several machines with discrete speeds and an induction-excited alternator. He also produced slow high-torque motors using the direct pull between magnetised surfaces.

Whatever his interest at a particular time, Professor Williams spent practically all his time in the laboratory or the workshop. Administrative work was left as much as possible to others and the time spent at his desk was minimised. He was nearly always to be found, in a cloud of tobacco smoke, supervising the construction of a device or its testing.

He was awarded honorary doctorates from four Universities and was elected to Fellowship of the Royal Society in 1950. He received many medals and other awards. He was made a Knight Bachelor in the Birthday Honours in 1976. In his own University he was held in great respect and affection and he ran his Department with an easy authority; his staff pursued their own interests and under his guidance their individual abilities developed. More than 20 of his protégés are now Professors.

Sir Frederic is survived by Lady Williams, a son, a daughter and four grandsons.

L. S. Piggott

## Reports and Publications

### UK and Ireland—September

- Moon, Mars and Meteorites. Pp. 36. (London: HMSO, 1977. Published for the Institute of Geological Sciences.) 70p net. [310]
- International Planned Parenthood Federation. Occasional Essay No. 5: Health, Nutrition and Population in Human Settlements. By Fred T. Sai. Pp. 32. (London: International Planned Parenthood Federation, 18-20 Lower Regent Street, SW1, 1977.) 85p; \$1.45. [310]
- Health and Safety Commission. Report 1974-76. Pp. 51. (London: HMSO, 1977.) £2 plus postage. [310]
- National Institute of Agricultural Botany. Fifty-Seventh Report and Accounts, 1976. Pp. 110. (Cambridge: National Institute of Agricultural Botany, 1977.) £1.25 net. [310]
- Office of Population Censuses and Surveys. Mortality Statistics: Accidents and Violence. (Review of the Registrar General on Deaths Attributed to Accidental and Violent Causes in England and Wales, 1975.) (Series DH4 No. 2) Pp. viii+37. (London: HMSO, 1977.) £1.25 net. [310]
- Thames Water Statistics—1976. Vol. 2: Water Quality Statistics, 1974/75/76. (London: Thames Water Authority, New River Head, Roseberry Avenue, EC1, 1977.) [310]
- University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1976-1977. (Supplement (3) to *Oxford University Gazette*, No. 3707.) Pp. 39-61. (Oxford: The University, 1977.) 20p. [310]
- Oystercatchers and Shellfish: Predator/Prey Studies. By John Goss-Custard, Selwyn McGrorty and Chris Reading. Pp. 10. (Cambridge: Institute of Terrestrial Ecology, Natural Environment Research Council, 1977.) 60p net. [410]
- Memoirs of the Royal Astronomical Society. Vol. 84, Part 2: The Luminosity Distribution of Globular Clusters in the Virgo Cluster of Galaxies. By David A. Hanes. Observations of 104 Extragalactic Radio Sources with the Cambridge 5-km Telescope at 5 GHz. By C. J. Jenkins, G. G. Pooley and J. M. Riley. A Search for Beta Canis Majoris Stars. By L. A. Balona. Radial Velocities of Southern B Stars Determined at the Radcliffe Observatory—VIII. Stars with HD Spectral Types B8 and B9. By Roger Wood. Pp. 45-134. (Oxford and London: Blackwell Scientific Publications, 1977. Published for the Royal Astronomical Society.) [510]
- Green Crop Fractionation. (Proceedings of a Symposium organised by the British Grassland Society and the British Society of Animal Production, 25-26 November, 1976, held at Harrogate, Yorkshire.) Edited by R. J. Wilkins. Pp. vii+189. (Occasional Symposium No. 9.) (Hurley, Maidenhead: British Grassland Society, 1977.) £5. [610]
- Department of Health and Social Security. Nutrition Education: Report of a Working Party. (British Nutrition Foundation. Department of Health and Social Security. Health Education Council.) Pp. v+30. (London: Department of Health and Social Security, 1977. Obtainable from HMSO.) [610]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1342: The Interaction of Large Amplitude Barotropic Waves with an Ambient Shear Flow: Critical Flows. By E. Varley, J. Y. Kazakia and P. A. Blythe. Pp. 189-236. UK £2.90; Overseas £3. Vol. 287, No. 1343: Semi-Classical Mechanics in Phase Space: a Study of Wigner's Function. By M. V. Berry. Pp. 237-271. UK £2.10; Overseas £2.20. (London: The Royal Society, 1977.) [610]
- Meteorological Office. Geophysical Memoirs No. 120: Average Temperatures, Contour Heights and Winds at 30 Millibars Over the Northern Hemisphere. By R. A. Ebdon. Pp. v+170 (48 plates). (London: HMSO, 1977.) £11.50 net. [710]
- Annual Report of the Soil Survey, England and Wales, 1976. Pp. 43. (Harpenden, Herts: The Soil Survey, Rothamsted Experimental Station, 1977.) [1010]
- Royal Observatory Annals. No. 11: Photoheliographic Results 1967. Pp. 114. (Herstmonceux Castle, Hailsham, Sussex: Royal Greenwich Observatory, 1975.) £2.65 net. [1010]
- Progress in Crystal Growth and Characterization, Vol. 1, No. 1, 1977. Edited by Dr. Brian R. Pamplin. Pp. 1-91. Published as one volume of four parts per year. 1977 Subscription rate: \$45. (Oxford: Pergamon Press, Ltd., 1977.) [1110]
- Medical Research Council. Senile and Presenile Dementias: a Report of the MRC Subcommittee. Compiled by W. A. Lishman. Pp. iv+23. (London: MRC, 1977.) [1310]
- Rheumatology Workshop: a Modern Review of Geigy Pyrazoles. (Proceedings of a Meeting held at Grand Hotel Verdala, Rabat, Malta, G. C., 9th-13th March 1977.) (*The Journal of International Medical Research*, Vol. 5, Supplement 2.) Pp. 120. Symposium on Danol (Danazol), held at The Royal College of Physicians, London, England, on 29th April 1977. (*The Journal of International Medical Research*, Vol. 5, Supplement 3.) Pp. 127. (Northampton: Cambridge Medical Publications, Ltd., 1977.) [1310]
- Department of the Environment. Welsh Office. Development Control Statistics 1975-76. Pp. v+44. (London: Department of the Environment, 20 Albert Embankment, 1977.) £1. [1310]
- Progress in Nuclear Energy, Vol. 1, No. 1, New Series. Executive Editors: M. M. R. Williams and R. Sher. Pp. 1-71. (Oxford and New York: Pergamon Press, 1977.) £10.50. [1310]
- Radiating Cosmic Dust. By C. D. Andriess. Pp. 107-190. (*Vistas in Astronomy*, Vol. 21, Part 2.) (Oxford and New York: Pergamon Press, 1977.) £8.30. [1310]
- Withdrawals of Authorisations for the use of NHS Hospital Accommodation and Services by Private Patients. (Proposals made by the Health Services Board under Section 4 of the Health Services Act 1976.) Pp. 23. (London: HMSO, 1977.) 45p net. [1410]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1344: The Instability of the Thin Vortex Ring of Constant Vorticity. By Sheila E. Widnall and Chong-Yin Tsai. Pp. 273-305 + plate 1. (London: The Royal Society, 1977.) UK £2.15; Overseas £2.25. [1710]
- Welsh Plant Breeding Station. Annual Report for 1976. Pp. 216. (Plas Gogerddan, Near Aberystwyth: Welsh Plant Breeding Station, University College of Wales, 1977.) £2. [1910]
- Native Pinewood of Scotland: Proceedings of Aviemore Symposium, 1975. Edited by Dr. R. G. H. Bunce and Mr. J. N. R. Jeffers. Pp. x+120. Cambridge: Natural Environment Research Council, Institute of Terrestrial Ecology, 68 Hills Road, 1977.) £2.50 net. [2010]
- Outlook, No. 1, Autumn 1976. (Published Bi-Annually for Open University graduates.) Pp. 1-16. (Walton Hall, Milton Keynes: The Open University, 1977.) [2010]
- Proceedings of the Royal Society of London. B: Biological Sciences. Vol. 199, No. 1134: A Discussion on Technologies for Rural Health. Organised by D. A. J. Tyrrell, F. R. S., D. P. Burkitt, F. R. S., and Sir William Henderson, F. R. S. Pp. 1-187. (London: The Royal Society, 1977.) UK £5.10; Overseas £5.30. [2010]
- National Institute for Medical Research. Report for 1976-77. Pp. iii+122. (London: Medical Research Council, 1977.) [2010]
- University of London. University College Calendar 1977-78. Pp. 186. (London: University College, 1977.) [2110]
- Department of Health and Social Security. Smoking and Professional People. Pp. 10. (London: Department of Health and Social Security, 1977. Available from HMSO.) [2410]
- Department of Industry. Changes in the Population of Persons with Qualifications in Engineering, Technology and Science, 1959 to 1976. (Studies in Technological Manpower, No. 6.) Pp. viii+51. (London: HMSO, 1977.) £3.25 net. [2410]
- International Union of Pure and Applied Chemistry. Physicochemical Measurements: Catalogue of Reference Materials from National Laboratories. Pp. 505-515. (Oxford and New York: Pergamon Press, 1977.) £3.30. [2610]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 280, No. 975: Pleistocene History of the Vale of St. Albans. By P. L. Gibbard. Pp. 445-483. UK £3.25; Overseas £3.35. Vol. 280, No. 976: The Structure of Plankton Communities. By J. H. Steele and B. W. Frost. Pp. 435-534 UK £3.50; Overseas £3.60. Vol. 280, No. 977: Some New Namurian Bivalve Faunas and Their Significance in the Origin of Carbonicola and in the Colonization of Carboniferous Deltaic Environments. By R. M. C. Eager. Pp. 535-570 + plates 1-4. UK £2.95; Overseas £3.05. (London: The Royal Society, 1977.) [2610]
- Snowdon. Pp. 7. (Cheltenham: Countryside Commission, John Dower House, Crescent Place, 1977.) gratis. [3110]

### Other Countries—October

- Centre National pour l'Exploitation des Océans. Rapport Annuel 1976. Pp. 87. (Paris: Centre National pour l'Exploitation des Océans, 1977.) [310]
- Institut National de la Recherche Agronomique. Service d'Etude des Sols et de la Carte Pédologique de France. Carte Pédologique de France à 1:100,000—Dijon. Notice Explicative par J. Chrétien. Pp. 218. (Versailles: Centre National de Recherches Agronomiques, 1976.) F.53.50. [310]
- Republic of South Africa: Department of Industries. Sea Fisheries Branch Investigational Report No. 113: Stock Differentiation and Growth of the Southern African Kingfish *Gerytherus capensis*. By A. I. L. Payne. Pp. 32. (Sea Point, Cape Town: Sea Fisheries Branch, Department of Industries, 1977.) [510]
- United States Department of the Interior: Geological Survey. Professional Paper 994-A: Depositional Environment of Upper Cretaceous Black Sandstones of the Western Interior. By Robert S. Houston and John F. Murphy. Pp. v+29. (Washington, DC: US Government Printing Office, 1977.) [510]
- Siemens Forschungs- und Entwicklungsberichte/Research and Development Reports, 5/77, Bd. 6. Edited by Hans Suchlandt. Pp. 263-321. (Berlin and New York: Springer-Verlag, 1977.) [610]
- United States Department of the Interior: Geological Survey. Bulletin 1278-E: Geochemical Exploration Techniques Based on Distribution of Selected Elements in Rocks, Soils, and Plants, Vekol Porphyry Copper Deposit Area, Pinal County, Arizona. By Maurice A. Chaffee. Pp. v+78. (Washington, DC: US Government Printing Office, 1977.) [610]
- Indian Council of Medical Research. Annual Report of the Director-General, 1976: Pp. 168. (New Delhi: Indian Council of Medical Research, 1977.) [610]
- Swedish College of Forestry. Studia Forestalia Suecica, No. 137: Observations on Trees of Scots Pine (*Pinus sylvestris* L.) and Lichens Around an HF and SO<sub>2</sub> Emission Source. By Jan-Erik Håggren and Bengt Nyman. Pp. 40. Nr. 138: Site Index Estimation by Means of Site Properties, Scots Pine and Norway Spruce in Sweden. By Björn Håggren and Jan-Erik Lundmark. Pp. 38. Nr. 139: Isozyme Studies in Seed Orchards. By Dag Rudin and Dag Lindgren. Pp. 23. (Stockholm: Swedish College of Forestry, 1977. Obtainable from Liber Distribution, S-162 89 Vällingby, Sweden.) [610]
- CERN—European Organization for Nuclear Research. CERN 77-16: K<sup>-</sup> and K<sup>-</sup>p Elastic Scattering in K<sup>-</sup>d Collisions from 1.2 to 2.2 GeV/c. By Y. Declais, J. Duchon, M. Louvel, J.-P. Patry, J. Seguinot, P. Bailon, C. Bricman, M. Ferro-Luzzi, J.-M. Perraut and

- T. Ypsilantis. Pp. vi+86. (Geneva: CERN, 1977.) [710]
- Marijuana Research Findings: 1976. Edited by Robert C. Petersen. (NIDA Research Monograph 14.) (US Department of Health, Education and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration.) Pp. vi+251. (Washington, DC: US Government Printing Office, 1977.) [1010]
- Department of Health, New Zealand. Environmental Radioactivity—Annual Report 1976. Pp. 13. (Christchurch: National Radiation Laboratory, 1977.) [1010]
- Smithsonian Contributions to Zoology, No. 239: Comparative Ethology of the Large-spotted Genet (*Genetta tigrina*) and Some Related Viverrids. By Christen M. Wemmer. Pp. iii+93. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [1010]
- National Institute on Drug Abuse. Research Monograph Series, No. 13: Cocaine—1977. Edited by Robert C. Petersen and Richard C. Stillman. Pp. 223. (Rockville, Md.: Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, 1977. For sale by US Government Printing Office, Washington, DC.) \$3. [1310]
- Fluid Phase Equilibria, Vol. 1, No. 1, September 1977. Pp. 1-91. Subscription for 1977/78: \$58.95; Dfl. 144. Free sample copies available. (Amsterdam: Elsevier Scientific Publishing Company, 1977.) [1310]
- Intelligence, Vol. 1, No. 1, January 1977. Edited by Douglas Detterman. Pp. 1-125. Published quarterly. Subscription Vol. 1, 1977: \$38; Personal Subscription \$15. (Norwood New Jersey: Ablex Publishing Corporation, 1977.) [1410]
- Cognitive Science, Vol. 1, No. 1, January 1977. Edited by Roger Schank, Allan Collins and Eugene Charniak. Pp. 1-123. Published quarterly. Subscription, Vol. 1, 1977: \$38; Personal subscription \$15. (Norwood, New Jersey: Ablex Publishing Corporation, 1977.) [1410]
- Smithsonian Contributions to Zoology, No. 252: *Anisochromia strausi*, New Species of Protogynous Hermaphroditic Fish, and Synonymy of *Anisochromidae*, *Pseudoplesiopidae*, and *Pseudochromidae*. By Victor G. Springer, C. Lavett Smith, and Thomas H. Fraser. Pp. 15. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [1410]
- A Report on the Lawrence Berkeley Laboratory. Pp. 67. (Berkeley, Calif.: Lawrence Berkeley Laboratory, University of California, 1977.) [1410]
- International Development Research Centre, Ottawa. On Common Ground: Report on the activities of IDRC 1976/1977. Pp. 30. (Ottawa: IDRC, 1977.) [1710]
- United States Department of the Interior: Geological Survey. Professional Paper 1020: Ordovician and Silurian Graptolite Succession in the Trail Creek Area, Central Idaho—a Graptolite Zone Reference Section. By Claire Carter and Michael Churkin, Jr. Pp. iv+36 + plates 1-7. (Washington, DC: US Government Printing Office, 1977.) [1710]
- CERN: European Organization for Nuclear Research. CERN 77-15: ISR Performance for Pedestrians. By K. Hübner. Pp. 55. (Geneva: CERN, 1977.) [1710]
- Australian Institute of Marine Science. Monograph Series, Vol. 1: Scleractinia of Eastern Australia. Part 1. Families Thamasteriidae, Astrocoeniidae, Pocilloporidae. By J. E. N. Veron and Michel Pichon. Pp. 86. (Townsville, Qd.: Australian Institute of Marine Science, 1976.) A\$4.70 plus postage. [1810]
- The Study of the Future: An Agenda for Research. Edited by Wayne I. Boucher. With contributions by Roy Amara et al. Pp. ix+316. (Washington, DC: National Science Foundation, 1977. Obtainable from US Government Printing Office, Washington.) \$4.75. [2010]
- The Pasture Institute of Southern India, Coonoor. Annual Report of the Director 1975, and Scientific Report 1976. Pp. 68. (Coonoor, S. India: The Pasture Institute of Southern India, 1977.) [2010]
- Smithsonian Contributions to Paleobiology, No. 33: Evolution of *Oblitacystis* from *Paleocystis* (Ostracoda: Trachyleberididae) During the Cenozoic in the Mediterranean and Atlantic. By Richard H. Benson. Pp. iii+47. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [2010]
- Government of India. Annual Report of the Department of Space, 1976/1977. Pp. 64. (Bangalore: Department of Space, Government of India, 1977.) [2110]
- Etude de l'Accélération de la Diffusion dans l'Or et dans l'Aluminium sous Irradiation Neutronique. Par Denis Acker. (These présentée à l'Université de Paris. Sud pour obtenir le Grade de Docteur es Sciences.) Pp. 154. (C. E. N. de Saclay, B. P. No. 2, 91190 Gif-sur-Yvette: Commissariat à l'Energie Atomique, Division de Métallurgie et d'Etude des Combustibles Nucléaires, 1977.) [2110]
- National Science Council, Taipei, Taiwan. NSC Review, 1975-76. Pp. 136. (Taipei, Taiwan: National Science Council, Republic of China, 1977.) [2410]
- World Health Organization. Technical Report Series. No. 611: Use of Ionizing Radiation and Radionuclides on Human Beings for Medical Research, Training, and Nonmedical Purposes—Report of a WHO Expert Committee. Pp. 39. (Geneva: WHO; London: HMSO, 1977.) Sw. fr. 6; \$2.40. [2410]
- Australia: Commonwealth Scientific and Industrial Research Organization. CSIRO Plant Industry 1976. Pp. 146. (East Melbourne: CSIRO, 1977.) [2410]
- Australian Journal of Zoology, Supplementary Series. No. 50: The Generic Classification of the Bolboceratini of the Australian Region, with Descriptions of Four New Genera (Scarabaeidae: Geotrupinae). By H. F. Howden and J. B. Cooper. Pp. 50. No. 51: A Revision of Australian Fanniidae (Diptera: Calyptrata). By A. C. Pont. Pp. 60. (East Melbourne: Editorial and Publications Service, CSIRO, P.O. Box 89, 1977.) [2410]
- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Animal Health, 1976. Pp. 160. (East Melbourne: CSIRO, 1977.) [2410]



## Recent scientific and technical books

## Mathematics

- ALTMAN, M. *Contractors and Contractor Directions: Theory and Applications—A New Approach to Solving Equations*. (Lecture Notes in Pure and Applied Mathematics, Vol. 32.) Pp. x + 200. ISBN-0-8247-6672-5. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr. 80.
- BAILEY, Jr., W. L., and SHIODA, T. (edited by). *Complex Analysis and Algebraic Geometry*. (A Collection of Papers Dedicated to K. Kodaira.) Pp. xii + 401. ISBN-0-521-21777-6. (Cambridge, London and New York: Cambridge University Press, 1977.) £28.
- BERGER, Melvin S. *Nonlinearity and Functional Analysis: Lectures on Nonlinear Problems in Mathematical Analysis*. (Pure and Applied Mathematics: a Series of Monographs and Textbooks.) Pp. xix + 17. ISBN-0-12-090350-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$24.50; £17.40.
- BIGGS, Norman. *Interaction Models*. (Course given at Royal Holloway College, University of London, October–December 1976. London Mathematical Society Lecture Note Series, No. 30.) Pp. 101. ISBN-0-521-21770-9. (Cambridge, London and New York: Cambridge University Press, 1977.) £4.50.
- CHADWICK, P. *Continuum Mechanics: Concise Theory and Problems*. Pp. 174. ISBN-0-04-510057-8. (London: George Allen and Unwin, Ltd., 1976.) £3.95.
- DIEUDONNE, J. *Treatise on Analysis*, Vol. 5. Translated by I. G. Macdonald. (Pure and Applied Mathematics: a Series of Monographs and Textbooks, Vol. 10-V.) Pp. xii + 243. ISBN-0-12-215505-Xiv.5. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$21.50; £15.25.
- FIEBENBERG, Stephen E. *The Analysis of Cross-Classified Categorical Data*. Pp. x + 151. ISBN-0-262-06063-9. (Cambridge, Mass. and London: the MIT Press, 1977.) \$10.95.
- GIBBONS, Jean Dickinson, OLKIN, Ingram, and SOBEL, Milton. *Selecting and Ordering Populations: A New Statistical Methodology*. (A Wiley Publication in Applied Statistics.) Pp. xxi + 569. ISBN-0-471-02670-0. (New York and London: John Wiley and Sons, 1977.) \$31.70; £18.75.
- GONZALEZ, Rafael, and WINTZ, Paul. *Digital Image Processing*. (Applied Mathematics and Computation, No. 13.) Pp. xvi + 431. ISBN-0-201-02597-3. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1977.) Hard binding \$29.50; Paper binding \$19.50.
- GREEN, J. R., and MARGERISON, D. *Statistical Treatment of Experimental Data*. Pp. x + 382. ISBN-0-444-41615-3. Amsterdam: Elsevier Scientific Publishing Company, 1977. Distributed in the USA and Canada by Elsevier North-Holland, Inc., New York.) Dfl. 85; \$34.95.
- IYANAGA, S., and KAWADA, Y. (edited by). *Encyclopedic Dictionary of Mathematics*. By the Mathematical Society of Japan. Translation reviewed by Kenneth O. May. Vol. 1: xiv + 1-883. Vol. 2: Pp. 885-1750. ISBN-0-262-09016-3. (Cambridge, Mass. and London: The MIT Press, 1977.) £80.
- MASON, J. David (edited by). *Proceedings of the Conference on Stochastic Differential Equations and Applications*, Park City, Utah, February 17-20, 1976. Pp. ix + 253. ISBN-0-12-478050-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$10.50; £7.45.
- MICCHELLI, Charles A., and RIVLIN, Theodore J. (edited by). *Optimal Estimation in Approximation Theory*. (The IBM Research Symposia Series.) Pp. ix + 300. ISBN-0-306-31049-X. (New York and London: Plenum Press, 1977.) \$35.40.
- RABINOWITZ, Paul H. (edited by). *Applications of Bifurcation Theory*. (Proceedings of an Advanced Seminar Conducted by the Mathematics Research Center, The University of Wisconsin at Madison, October 27-29, 1976. Publication No. 38.) Pp. ix + 389. ISBN-0-12-574250-9. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$15.50; £11.
- SZMYDYT, Zofia. *Fourier Transformation and Linear Differential Equations*. Translated from the Polish by Marcin E. Kucznik. Pp. xix + 503. ISBN-90-277-0622-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, Warszawa: PWN—Polish Scientific Publishers, 1977.) Dfl. 90; \$34.
- VAN RYZIN, J. (edited by). *Classification and Clustering*. (Proceedings of an Advanced Seminar Conducted by the Mathematics Research Center, The University of Wisconsin at Madison, May 3-5, 1976.) Pp. x + 467. ISBN-0-12-714250-9. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$17; £12.05.

## Astronomy

- COLLINS, Mike (compiled by). *Astronomical Catalogues 1951-1975*. (Bibliography Series No. 2.) Pp. ix + 325. ISBN-0-85296-440-4. (Hitchin, Herts.: INSPEC, The Institution of Electrical Engineers, 1977.) UK £70; rest of the world £80.
- DE JONG, T., and MAEDER, A. (edited by). *Star Formation*. (International Astronomical Union Astronomique Internationale, Symposium No. 75, held in Geneva, Switzerland, September 6-10, 1976.) Pp. xiv + 296. ISBN-90-277-0796-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Cloth Dfl. 75; \$30. Paper Dfl. 45; \$18.
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- MITTON, Simon (editor-in-chief). *The Cambridge Encyclopedia of Astronomy*. Pp. 481. ISBN-0-224-01418-8. (London: Jonathan Cape, Ltd., 1977.) £15 net.
- MÜLLER, Edith (edited by). *Highlights of Astronomy*, Vol. 4. (As presented at the 16th General Assembly, 1976. International Astronomical Union Astronomique Internationale.) Part 1: Pp. 370. ISBN-90-277-0849-5. Cloth Dfl. 85; \$34. Paper Dfl. 55; \$22. Part 2: Pp. 403. ISBN-90-277-0850-9. Cloth Dfl. 95; \$38. Paper Dfl. 62.50; \$25. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.)
- MÜLLER, Edith A., and JAPPEL, Arnold (edited by). *Proceedings of the Sixteenth General Assembly, Grenoble 1976*. (International Astronomical Union Astronomique Internationale. Transactions of the International Astronomical Union, Vol. 16B.) Pp. x + 586. ISBN-90-277-0836-3. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl. 125; \$50.
- ROWAN-ROBINSON, Michael. *Cosmology*. (Oxford Physics Series.) Pp. x + 158. ISBN-0-19-851838-2. (Oxford: Clarendon Press, London: Oxford University Press, 1977.) Boards £6 net. Paper £2.95 net.

## Physics

- BRAGINSKY, V. B., and MANUKIN, A. B. *Measurement of Weak Forces in Physics Experiments*. Edited by David H. Douglass. Pp. xiii + 153. ISBN-0-226-07070-0. (Chicago and London: The University of Chicago Press, 1977.) £7.
- CHEN, Chih-Wen. *Magnetism and Metallurgy of Soft Magnetic Materials*. (Series of Monographs on Selected Topics in Solid State Physics.) Pp. xviii + 571. ISBN-0-7204-0706-0. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 175; \$71.50.
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- DUGDALE, J. S. *The Electrical Properties of Metals and Alloys*. (The Structures and Properties of Solids, 5.) Pp. 292. ISBN-0-7131-2524-1. (London: Edward Arnold (Publishers), Ltd., 1977.) Boards £10.50; Paper £4.95.
- FLUENDY, M. A. D. *Chemical Applications of Molecular Beam Scattering*. (Studies in Chemical Physics.) Pp. xi + 400. ISBN-0-412-15550-8. (London: Chapman and Hall, 1977.) Distributed in the USA by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) £5.50.
- HENDERSON, B., and WERTZ, J. E. *Defects in the Alkaline Earth Oxides, with Applications to Radiation Damage and Catalysis*. (Taylor and Francis Monographs on Physics.) Pp. 159. ISBN-0-85066-086-6. (London: Taylor and Francis, Ltd., 1977.) £8.50 net.
- KALDIS, E., and SCHEEL, H. J. (edited by). *1976 Crystal Growth and Materials*. (A Selection

of Review Papers Presented at the First European Conference on Crystal Growth ECCG-I and Materials Symposium, Zurich, Switzerland, 12-18 September 1976.) (Current Topics in Materials Science, Vol. 2.) Pp. xvi + 916. ISBN-0-7204-0740-0. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 300; \$122.50.

KANTOR, Frederick W. *Information Mechanics*. Pp. xiii + 397. ISBN-0-471-02968-8. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$27.90; £16.45.

KNOEPFEL, H. (edited by). *Tokamak Reactors for Breakeven: a Critical Study of the Near-Term Fusion Reactor Program*. Erice-Trapani (Sicily), September 21-October 1, 1976. (International School of Fusion Reactor Technology, "Ettore Majorana" Centre for Scientific Culture.) Pp. 659. ISBN-0-08-022034-7. (Oxford and New York: Pergamon Press, 1978. Published for the Commission of the European Communities.) £30.50.

LEVY, R. A., and HASEGAWA, R. (edited by). *Amorphous Magnetism II*. Pp. xii + 680. ISBN-0-306-34412-2. (New York and London: Plenum Press, 1977.) \$71.40.

LIETH, R. M. A. (edited by). *Preparation and Crystal Growth of Materials with Layered Structures*. (Physics and Chemistry of Materials with Layered Structures, Vol. 1.) Pp. viii + 280. ISBN-90-277-0638-7. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl. 95; \$38.

McCAIG, Malcolm. *Permanent Magnets in Theory and Practice*. Pp. 374. ISBN-0-7273-1604-4. (London and Plymouth: Pentech Press, 1977.) £12.50.

SAXE, Robert L. *The Chain Photon Theory of Matter and Energy*. Pp. xxii + 340. (New York: Robert L. Saxe, 1977.) np.

SHARP, Robert T., and KOLMA, Bernard (edited by). *Group Theoretical Methods in Physics*. (Proceedings of the Fifth International Colloquium, Université de Montreal, July 1976.) Pp. xvi + 668. ISBN-0-12-637650-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$29.50; £20.95.

SPEAR, W. E. (edited by). *Amorphous and Liquid Semi-conductors*. (Proceedings of the Seventh International Conference, Edinburgh, June 27-July 1, 1977.) Pp. xv + 891. (Edinburgh: Centre for Industrial Consultancy and Liaison, University of Edinburgh, 1977. Published on behalf of the Conference Committee.) £37.50.

## Chemistry

ANDEREGG, G. (prepared for publication by). *Critical Survey of Stability Constants of EDTA Complexes*. (International Union of Pure and Applied Chemistry, Analytical Chemistry Division. IUPAC Chemical Data Series, No. 14.) Pp. 42. ISBN-0-08-022009-6. (Oxford and New York: Pergamon Press, 1977.) £3.90.

BARNES, A. J., ORVILLE-THOMAS, W. J. (edited by). *Vibrational Spectroscopy—Modern Trends*. Pp. xii + 442. ISBN-0-444-41632-3. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 122; \$49.95.

BERNE, Bruce J. (edited by). *Statistical Mechanics, Part B: Time-Dependent Processes*. (Modern Theoretical Chemistry, Vol. 6.) Pp. xv + 362. ISBN-0-306-33506-9(v. 6). (New York and London: Plenum Press, 1977.) \$47.40.

BRAME, Jr., Edward F., and GRASELLI, Jeanette G. (edited by). *Infrared and Raman Spectroscopy, Part C*. Pp. ix + 717-1039. ISBN-0-8247-6527-3. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr. 118.

Bu LOCK, J. D. (senior reporter). *Biosynthesis*, Vol. 5. (A Review of the Literature Published during 1975 and 1976.) (A Specialist Periodical Report.) Pp. ix + 318. ISBN-0-85186-543-7. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts.: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £22.50; \$45.

CAMPBELL, Ian M. *Energy and The Atmosphere: a Physical-Chemical Approach*. Pp. ix + 398. ISBN-0-471-09482-0. (London and New York: John Wiley and Sons, Ltd., 1977.) Cloth £14.50; \$31. Paper £5.95; \$12.50.

CRABBE, Pierre (edited by). *Prostaglandin Research*. (Organic Chemistry: a Series of Monographs, Vol. 36.) Pp. xv + 341. ISBN-0-12-194660-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$26; £18.45.

CROMPTON, T. R. *Chemical Analysis of Additives in Plastics*. Second edition. (International Series in Analytical Chemistry, Vol. 46.) Pp. xii + 366. ISBN-0-08-020497-X. (Oxford and New York: Pergamon Press, 1977.) £15.25.

DAY, P. (senior reporter). *Electronic Structure and Magnetism of Inorganic Compounds*, Vol. 5. (A Review of the Literature Published during 1974 and 1975. A Specialist Periodical Report.) Pp. viii + 248. ISBN-0-85186-291-8. (London: The Chemical Society, 1977. Obtainable from Chemical Society, Blackhorse Road, Letchworth, Herts.: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £21; \$42.

GUYOT, A. (symposium editor). *Polyvinylchloride—2*. (Main lectures presented at the Second International Symposium on Polyvinylchloride, Lyon-Villeurbanne, France, 5-9 July 1976.) Pp. 539-567. ISBN-0-08-021203-4. (Oxford and New York: Pergamon Press, 1977.) £13.50.

HANSON, J. R. (senior reporter). *Terpenoids and Steroids*, Vol. 7. (A Review of the Literature Published between September 1975 and August 1976. A Specialist Periodical Report.) Pp. x + 349. ISBN-0-85186-316-7. (London: The Chemical Society, 1977. Obtainable from Chemical Society, Blackhorse Road, Letchworth, Herts.: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £25; \$50.

JOLLY, William L. *The Principles of Inorganic Chemistry*. Pp. viii + 376. ISBN-0-07-032758-0. (New York and London: McGraw-Hill Book Company, 1976.) £12.75.

JONES, J. Bryan, SIH, Charles J., and PERLMAN, D. (edited by). *Applications of Biochemical Systems in Organic Chemistry*, Part 1: Pp. xii + 1-506 + 16. ISBN-0-471-93267-1, Vol. 2: Pp. x + 507-1065 + 16. ISBN-0-471-93270-1. (Techniques of Chemistry, Vol. 10.) (New York and London: Wiley-Interscience, John Wiley and Sons, 1976.) \$73.50; £43.50 the set.

KERK, Milton, ZETTEMAYER, Albert C., and ROWELL, Robert L. (edited by). *Colloid and Interface Science*, Vol. 1: Plenary and Invited Lectures. (Proceedings of the International Conference on Colloids and Surfaces—50th Colloid and Surface Science Symposium, held in San Juan, Puerto Rico on June 21-25, 1976.) Pp. xliii + 636. ISBN-0-12-404501-4 (v. 1). (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.)

KOWALSKI, Bruce R. (edited by). *Chemometrics: Theory and Application*. (A symposium sponsored by the Division of Computers in Chemistry at the 172nd Meeting of the American Chemical Society, San Francisco, Calif., Sept. 2, 1976.) Pp. xvii + 288. ISBN-0-8412-0379-2. (Washington DC: American Chemical Society, 1977.) \$21.

MANSKE, R. H. F. (edited by). *The Alkaloids: Chemistry and Physiology*, Vol. 16. Pp. xviii + 569. ISBN-0-12-469516-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$59; £41.90.

MILLER, R. Bryan, and WADE, Jr., L. G. (edited by). *Annual Reports in Organic Synthesis—1976*. Pp. xiv + 449. ISBN-0-12-040807-4. (New York and San Francisco: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$16.50; £11.70.

MITRA, Abhijit. *The Synthesis of Prostaglandins*. Pp. xiii + 444. ISBN-0-471-02308-6. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$28.60; £16.90.

MITTAL, K. L. (edited by). *Micellization, Solubilization, and Microemulsions*, Vol. 2. Pp. xv + 945. ISBN-0-306-31024-4(v. 2). (New York and London: Plenum Press, 1977.) \$54.

MOORE, Bradley (edited by). *Chemical and Biochemical Applications of Lasers*, Vol. 3. Pp. ix + 325. ISBN-0-12-505403-3(v. 3). (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$16.50; £11.70.

MUUS, L. T., ATKINS, P. W., McLAUCHLAN, K. A., and PEDERSEN, J. B. (edited by). *Chemically Induced Magnetic Polarization*. (Proceedings of the NATO Advanced Study Institute held at Sestria, Urbino, Italy, April 17-30, 1977.) (NATO Advanced Study Institutes Series, Series C: Mathematical and Physical Sciences, Vol. 34.) Pp. xi + 407. ISBN-90-277-0845-2. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977. Published in co-operation with Nato Scientific Affairs Division.) Dfl. 95; \$38.

NOTH, H. (symposium editor). *Boron Chemistry—3*. (Selected lectures presented at the Third International Meeting on Boron Chemistry, Munich and Ettal, FRG., 5-9 July 1976.) (International Union of Pure and Applied Chemistry, Inorganic Chemistry Division, in conjunction with the German Chemical Society.) Pp. 691-700. ISBN-0-08-021206-9. (Oxford and New York: Pergamon Press, 1977.) £9.75.

PEDLEY, J. B., and RYLAND, J. Sussex—N.P.L. *Computer Analysed Thermodynamic Data: Organic and Organometallic Compounds*. (Brighton: Dr. J. B. Pedley, School of Molecular Sciences, University of Sussex, 1977. Published with the permission of the Controller of Her Majesty's Stationery Office.) £10.

- PROCEEDINGS OF THE SYMPOSIUM ON FLUOROSIS, October 1974. Pp.xvi + 534. (Hyderabad, India: Indian Academy of Geoscience, 1977.) Rs.150; \$25; £10.
- PULLMAN, Bernard, and GOLDBLUM, Natan (edited by). Excited States in Organic Chemistry and Biochemistry. (Proceedings of the Tenth Jerusalem Symposium on Quantum Chemistry and Biochemistry held in Jerusalem, Israel, March 28/31, 1977.) (The Jerusalem Symposia in Quantum Chemistry and Biochemistry, Vol. 10.) Pp.xiii + 448. ISBN-90-277-0853-3. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl. 100; \$39.50.
- RASSER, J. C. Platinum-Iridium Reforming Catalysts: TPD of Hydrogen, Selectivity and Activity in Heptane Conversion. Pp.xiii + 216. ISBN-90-6275-005-2. (Delft: Delft University Press, 1977.) Dfl. 24.
- ROBERTS, M. W., and THOMAS, J. M. (senior reporters). Surface and Defect Properties of Solids, Vol. 6. (A Review of the Recent Literature published up to mid-1976. A Specialist Periodical Report.) Pp.x + 367. ISBN-0-85186-300-0. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £27; \$54.
- SANDLER, Stanley R., and KARO, Wolf. Polymer Syntheses, Vol. 2. (Organic Chemistry: a Series of Monographs, Vol. 29.) Pp.xi + 400. ISBN-0-12-618502-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$39; £28.05.
- SCOTT, R. P. W. Liquid Chromatography Detectors. (*Journal of Chromatography Library*, Vol. 11.) Pp.vii + 248. ISBN-0-444-41580-7. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 85; \$34.50.
- SEKINE, Tatsuya, and HASEGAWA, Yuko. Solvent Extraction Chemistry: Fundamentals and Applications. Pp.xii + 919. ISBN-0-8247-6391-2. (New York and Basel: Marcel Dekker, Inc., 1977.)
- SENNING, A., and MAGEE, P. S. (edited by). Topics in Sulfur Chemistry, Vol. 3. Pp. 128. ISBN-3-13-5263-01-0. (Stuttgart: Georg Thieme Publishers, 1977.) DM 58.
- SMETS, G. (symposium editor). Photochemical Processes in Polymer Chemistry—2. (Invited lectures presented at the Second IUPAC Symposium, Leuven, Belgium, 2-4 June, 1976.) (International Union of Pure and Applied Chemistry (Macromolecular Division), in conjunction with The University of Leuven.) Pp.403-538. ISBN-0-08-021205-0. (Oxford and New York: Pergamon Press, 1977.) £13.60.
- STOCKHAM, John D., and FOCHTMAN, Edward G. (edited by). Particle Size Analysis. Pp.xi + 140. ISBN-0-250-40189-4. (Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$26.40; £15.60.
- WEBB, G. A. (edited by). Annual Reports on NMR Spectroscopy, Vol. 7. Pp.ix + 300. ISBN-0-12-505307-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £16; \$31.25.
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## Technology

- AMANN, Ronald, COOPER, Julian, and DAVIES, R. W. With the assistance of JENKINS, Hugh. The Technological Level of Soviet Industry. Pp.xxii + 575. ISBN-0-300-02076-7. (New Haven and London: Yale University Press, 1977.) £20.
- BARTON, Edwin C. *et al.* (editorial publication board.) Advances in Automated Analysis: Technicon International Congress, 1976. Vol. 1: Clinical and Hospital Management Symposia. Pp.xxi + 490. Vol. 2: Industrial Symposia. Pp.xi + 366. (Tarrytown, NY: Mediad, Inc., 1977.)
- BOSS 76: Behaviour of Off-Shore Structures. (Proceedings of the First International Conference, held at The Norwegian Institute of Technology, The University of Trondheim, Norway, August 2nd-5th, 1976.) Vol. 1: Pp.1000. ISBN-0-08-021739-7. Vol. 2: Pp.676. ISBN-0-08-022154-8. (Oxford and New York: Pergamon Press, 1977.) £55.50 each volume.
- ECKERT, E. R. G., and IRVINE, Jr., Thomas F. (edited by). Progress in Heat and Mass Transfer, Vol. 8: Heat Transfer Reviews 1970-1975. (Monograph Series of the *International Journal of Heat and Mass Transfer*.) Pp.vii + 321. ISBN-0-08-021737-0. (Chicago: Rumford Publishing Company; Oxford and New York: Pergamon Press, 1977.) £19.
- GOERGE, Frank. Machine Takeover: The Growing Threat to Human Freedom in a Computer-Controlled Society. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xiii + 193. ISBN-0-08-021228-X. (Oxford and New York: Pergamon Press, 1977.) Hard £7.25; Flexi £3.50.
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- NEW INTERNATIONAL DICTIONARY OF REFRIGERATION: Multilingual. Pp.xxvii + 560. ISBN-0-08-020368-X. (Paris: Institut International du Froid/International Institute of Refrigeration, 1977. Distributed by Pergamon Press, Oxford.) £43.75.
- PAUL, J. K. Solar Heating and Cooling: Recent Advances. Pp.x + 485. ISBN-0-8155-0674-0. (Park Ridge, New Jersey: Noyes Data Corporation, 1977.) \$48.
- PERRY, Roger, and YOUNG, Robert J. (edited by). Handbook of Air Pollution Analysis. Pp.xiv + 506. ISBN-0-412-12660-5. (London: Chapman and Hall, 1977. Distributed in the USA by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) £20.
- RECENT ADVANCES IN MINING AND PROCESSING OF LOW-GRADE AND SUBMARGINAL MINERALS DEPOSITS. (Centre for Natural Resources, Energy and Transport, United Nations, New York.) Pp.192. ISBN-0-08-021051-1. (Oxford and New York: Pergamon Press, 1976.) £13.90; \$25.
- SCHULTZ, J. M. (edited by). Properties of Solid Polymeric Materials, Part A. (Treatise on Materials Science and Technology, Vol. 10.) Pp.xiv + 460. ISBN-0-12-341810-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$44; £31.25.
- SHAH, D. O., and SCHECHTER, R. S. (edited by). Improved Oil Recovery by Surfactant and Polymer Flooding. Pp.x + 578. ISBN-0-12-641750-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$23.50; £16.70.
- SILVERMAN, Joseph, and VAN DYKEN, A. R. (edited by). Radiation Processing. (Transactions of the First International Meeting on Radiation Processing, held at Dorado Beach, Puerto Rico, 9-13 May 1976.) Vol. 1: Invited Papers. Pp.xxvi + 1402. Vol. 2: Contributed Papers. Pp.xxviii + 403-885. ISBN-0-08-021640-4 (2 vol. set). (Oxford and New York: Pergamon Press, 1977.) £55.
- TEBBUTT, T. H. Y. Principles of Water Quality Control, Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.x + 201. ISBN-0-08-021296-4. (Oxford and New York: Pergamon Press, 1977.) Hardcover £7.50; Flexicover £3.75.
- THOMPSON-RUSSELL, K. C., and EDINGTON, J. W. Electron Microscope Specimen Preparation Techniques in Materials Science. (Philips Technical Library, Monographs in Practical Electron Microscopy in Materials Science, 5.) Pp.136. ISBN-0-333-21980-5. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) £9.
- YIH, Chia-Shun (edited by). Advances in Applied Mechanics, Vol. 17. Pp.vii + 389. ISBN-0-12-002017-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$43; £30.55.
- BARAGAR, W. R. A., COLEMAN, L. C. and HALL, J. M. (edited by). Volcanic Regimes in Canada. (Proceedings of a Symposium held at the University of Waterloo, Waterloo, Ontario, May 16-17, 1975.) (Special Paper No. 16.) Pp.viii + 476. (Waterloo, Ontario: Geological Association of Canada, Department of Earth Sciences, The University, 1977.)
- CARUTHERS, Jerald W. Fundamentals of Marine Acoustics. (Elsevier Oceanography Series, 18.) Pp.xii + 153. ISBN-0-444-41552-5. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 69; \$28.
- CHAPMAN, C. P. Human and Environmental Systems: a Geographer's Appraisal. Pp.xiv + 421. ISBN-0-12-168650-7. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £12.80; \$25.
- DOUGLAS, Ian. Humid Landforms. (An Introduction to Systematic Geomorphology, Vol. 1.) Pp.xvi + 288. ISBN-0-262-04054-9. (Cambridge, Mass.: The MIT Press, 1977.) \$15.
- GOUDIE, Andrew. Environmental Change. (Contemporary Problems in Geography.) Pp.xi + 244. ISBN-0-19-874073-5. (Oxford: Clarendon Press; London: Oxford University Press, 1977.) £7.50 net.
- GRANDAL, B., and HOLTET, J. A. (edited by). Dynamical and Chemical Coupling Between the Neutral and Ionized Atmosphere. (Proceedings of the NATO Advanced Study Institute, held at Spatind, Norway, April 12-22, 1977.) (NATO Advanced Institutes Series, Series C: Mathematical and Physical Sciences, Vol. 35.) Pp.xix + 392. ISBN-90-277-0840-1. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977. Published in co-operation with NATO Scientific Affairs Division.) Dfl. 90; \$35.
- HENIN, S. Cours de Physiques du Col. Tome II: L'eau et le Sol-Les Propriétés Mécaniques-La Chaleur et le Sol. (Initiations-Documents Techniques, No. 29.) Pp.221. ISBN-2-7099-0417-9. (Paris: ORSTOM; Bruxelles: EDITIONS, 1977.) Fr. 45.
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## Biology

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## Earth Sciences

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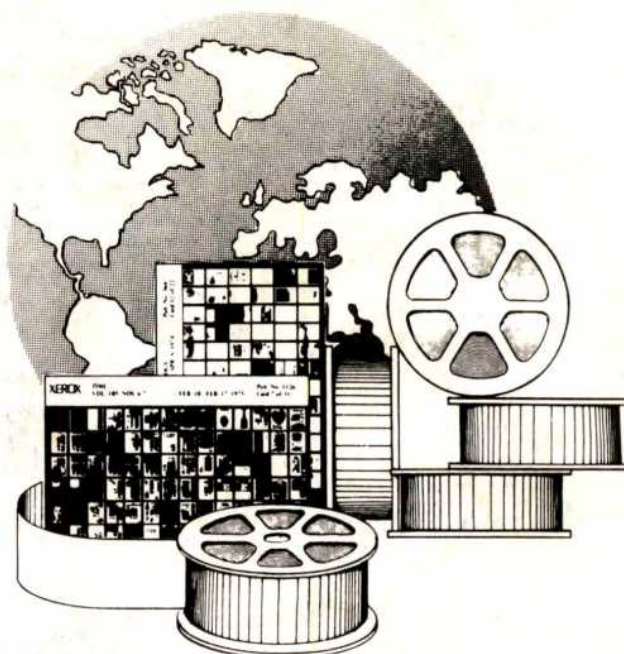
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